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SEX DETERMINATION

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SEX DETERMINATION

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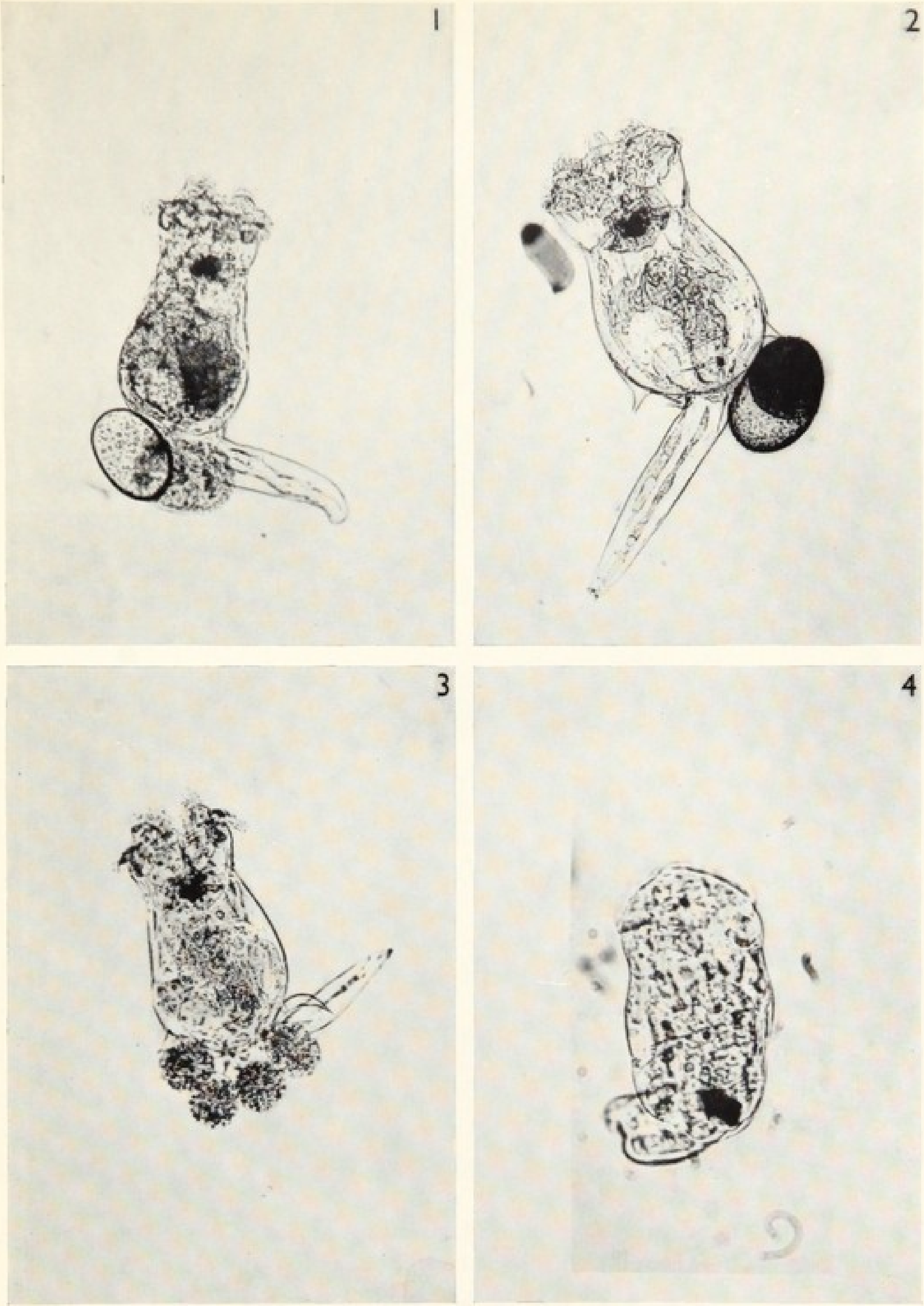

Plate I. The reproductive categories of a rotifer, *Brachionus* sp.

1. Amictic female with two subitaneous female eggs.
2. Mictic female with a single resting egg.
3. Mictic female with four male eggs.
4. Male.

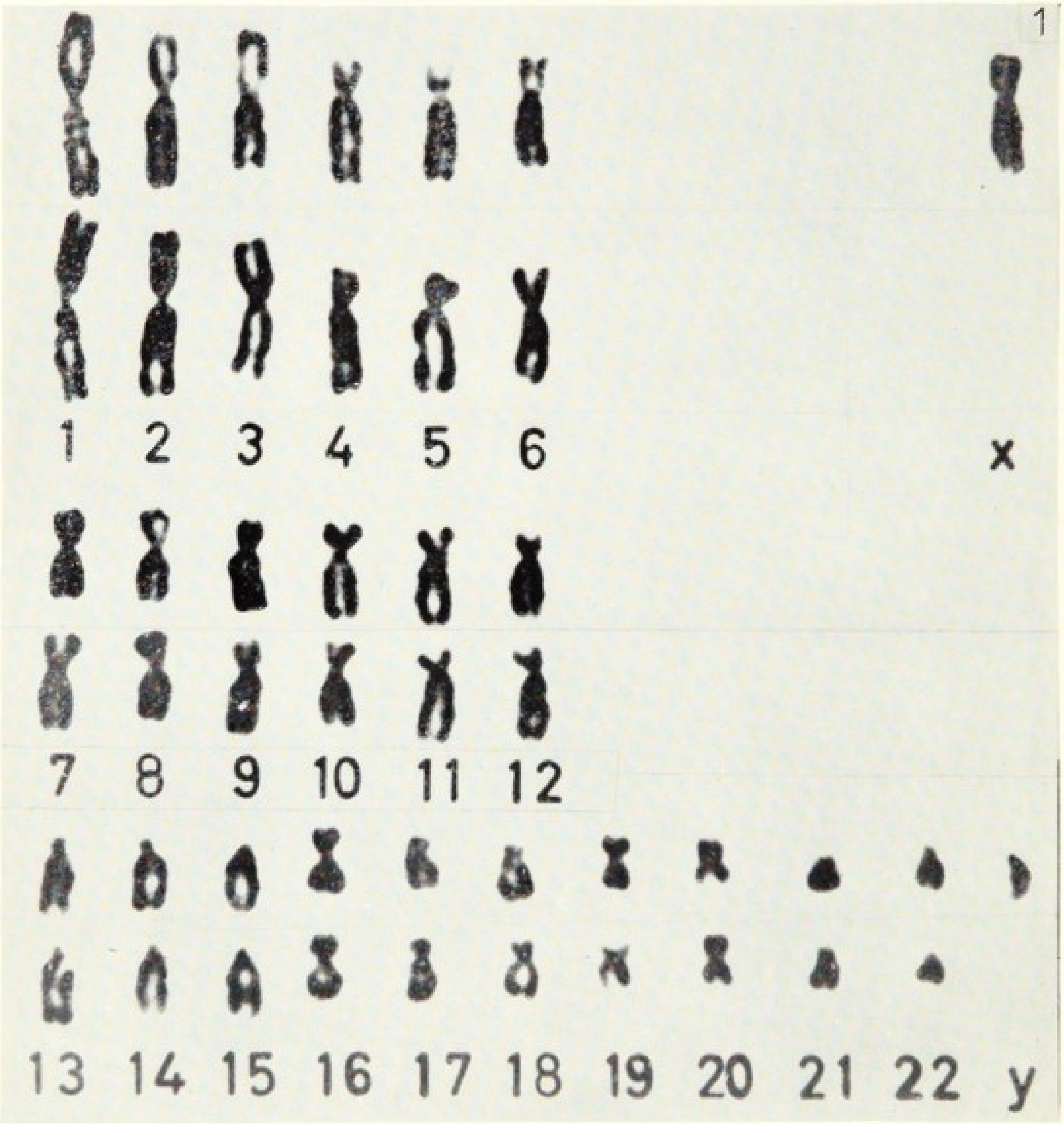

PLATE II.1. The mitotic chromosome of the human male according to the Denver system. (Reproduced by courtesy of Tjio and Puck, 1958, and *Proc. Nat. Acad. Sci.*)

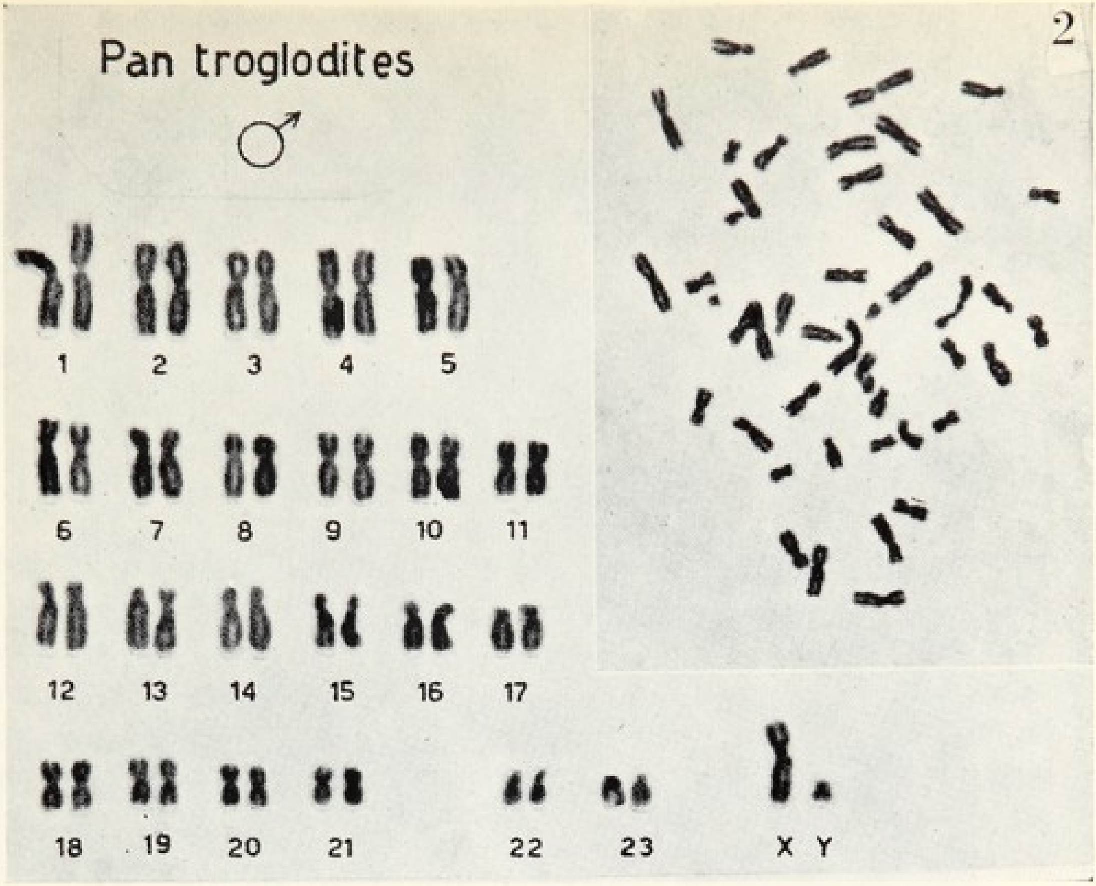

PLATE II.2. The mitotic chromosomes of the male chimpanzee, *Pan troglodites*. (Reproduced by courtesy of Chiarelli, 1962, and *Caryologia*)

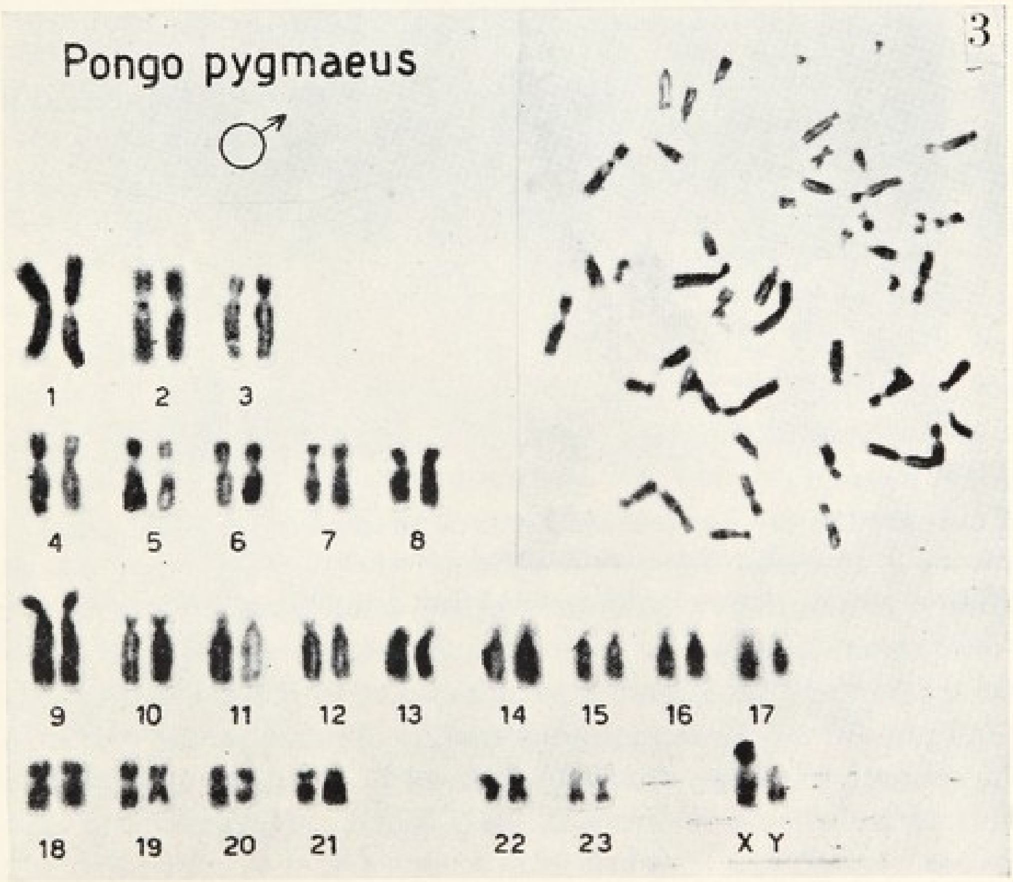

PLATE II.3. The mitotic chromosomes of the male orang, *Pongo pygmaeus*. (Reproduced by courtesy of Chiarelli, 1962, and *Caryologia*)

PLATE III. The human chromosome set as seen with a phase contrast microscope in an unstained bone marrow mitosis.

(Reproduced by courtesy of Powsner and Bermann, 1960, and *Nature*, London)

CHAPTER 1

SEXUAL AND ASEXUAL REPRODUCTION

Differentiation, Recombination and Sex Determination

THE word sex commonly conveys the idea of differentiation into the two opposed and complementary characters of maleness and femaleness. The shield and the spear ♂, the ancient alchemic symbols of iron and of Mars, and the mirror ♀, which was the symbol of copper and of Venus, indicate the poles which, according to many authors, are the primary features of sexuality. Careful investigation has shown that some kind of physiological bipolarity can be detected even in sexual reproductive cells that show no trace of morphological differences between the two sexes.

On the other hand increasing importance is being attached to the regular alternation of caryogamy and meiosis, a cycle that has proved to be a feature of all sexually reproducing organisms. Some authors simply identify sex with the union of gametes and with caryogamy (Lederberg, 1955) although it is one meiotic recombination which carries out amphimixis (Weissmann, 1886a), or the mixing of parental germ plasms, and is generally recognized as the process that confers sex its evolutionary significance.

Caryogamy and meiosis, with the accompanying process of recombination are therefore the essential features of the sexual cycle and differentiation is said to be constantly present before caryogamy takes place.

Researches on bacterial conjugation, on bacteriophage mediated transduction and on mitotic crossing over, which have been carried on in some bacterial strains and in several species of Fungi, have recently shown the existence of various possibilities for genetic recombination in the absence of a regular alternation of caryogamy and meiosis. The analysis of such highly aberrant, or extremely primitive, parasexual processes (Pontecorvo, 1954), whose very existence could only be established through the study of recombination, has led on the other hand to the detection of some peculiar types of differentiation, which curiously resemble sexual differences in higher organisms.

It was a matter of heated discussion during the early thirties whether sex union is possible without sex differentiation (Hartmann, 1932; Mainx, 1933) and a law stating the existence of an "allgemeine bipolare Zweigeschlechtlichkeit" was enunciated (Hartmann, 1943). It seems now sufficient to state that some kind of polarity—which may be identified with sex in its Aristotelian meaning—is generally shown by specialized stages of the reproductive cycles where genetic recombination has been demonstrated.

The problems of sex determination are concerned with the factors of the widespread—and maybe essential—phenomenon of sex differentiation and incidentally with some aspects of incompatibility in reproduction. It can be said that, from a general point of view, sex determination is obtained through the segregation of differences at meiosis. The mechanisms that lead to this result are extremely varied and may come into action at different stages of the sexual cycle.

It is important therefore to make a quick survey of the basic chromosomal and cytoplasmic processes that make possible both the unbroken transmission of heredity and the determination of individual and gametic sex diversity in the course of the reproductive cycle.

Reproduction and Chromosomes

The first decisive step to the modern understanding of reproductive processes was accomplished when it was realized that, since organisms are all composed of cells (Schleiden, Schwann), reproduction is necessarily linked with cellular division. The study of mitosis or karyokinesis (Schneider, Strassburger) rapidly lead to the discovery of the chromosomes (Waldeyer, 1890) and of their exact distribution in the nuclei of the daughter cells after mitosis.

1. Mitosis and its Significance in Heredity

The sequence of mitotic processes is now sufficiently known in its morphological aspects and it appears to take place in basically the same way in the cell divisions of all organisms, except bacteria.

The most impressive event occurring at the beginning of mitosis is the resolution within the nucleus of separate bodies which were called chromosomes because they were discovered, and are still generally examined, by employing peculiar basic stains. They were carefully studied from a morphological point of view as soon as their importance as bearers of hereditary factors was realized. Three main types of chromosomes have thus been described: telocentric or acrocentric, subtelocentric and metacentric. In the first type the chromosome is rodlike and the centromere is located very near to one of the ends, the subtelocentric chromosome has two unequal arms with the centromere on one side and the metacentric chromosome has two equal arms. Secondary constrictions, points of attachment of the nucleoli, regional differences in staining properties, and other minor peculiarities in form, behaviour and structure proved to be constant in individual chromosomes. The constancy of their basic number in each species is assured by the regularity of the mitotic process.

Mitosis is divided into the four stages of prophase, metaphase, anaphase and telophase but it must be remarked that direct observation on living material and motion pictures of cell divisions clearly demonstrate that the four phases are not sharply limited and that mitosis is a continuous process. The passage from the so-called resting stage or interphase, when the nuclei show little or ill-defined structure, to the early prophase is also very difficult to establish.

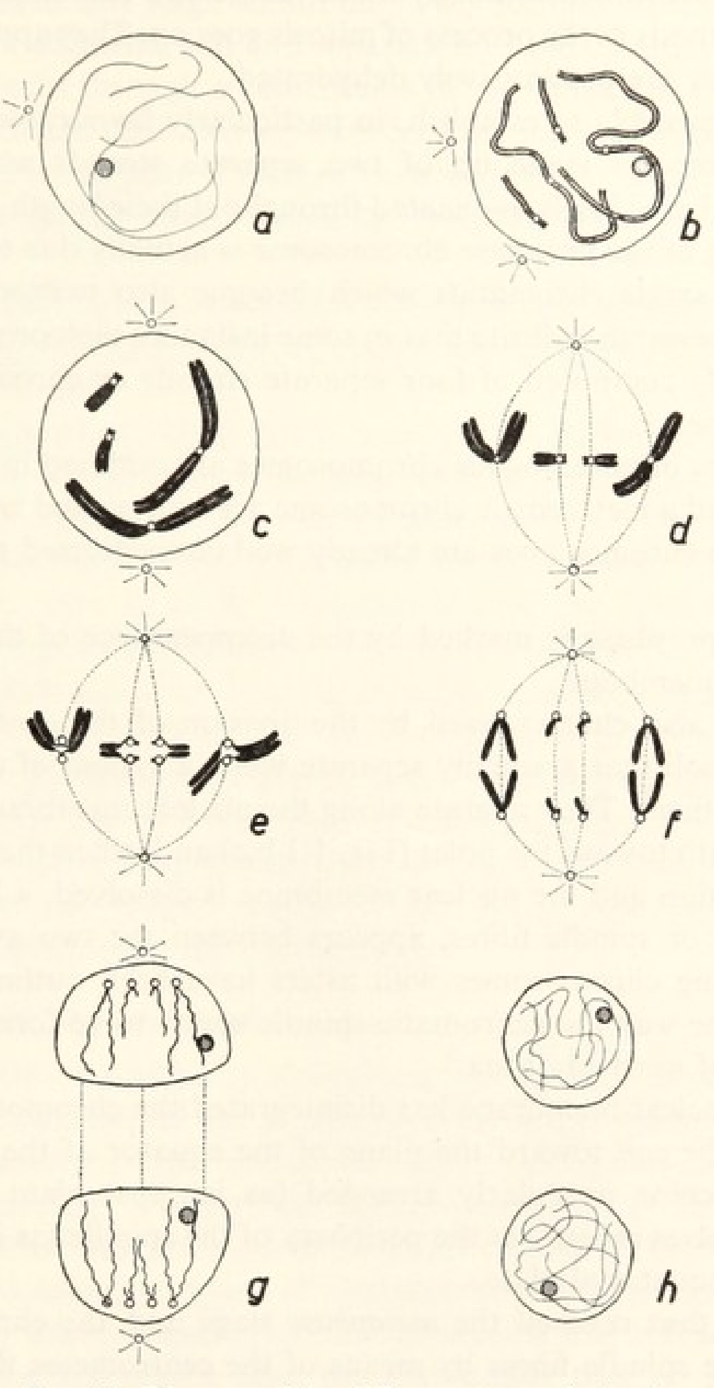

FIG. 1.1. Diagram of the different stages of mitosis.

In Fig. 1.1 is represented what is called a diploid set consisting of two pairs of similar or homologous chromosomes, the members of each pair being composed by one chromosome of paternal and by another chromosome of maternal origin.

At the beginning of *prophase* (Fig. 1.1a) chromosomes appear as delicate filaments that become increasingly more visible and stainable with the usual cytological methods as the process of mitosis goes on. They appear shorter and thicker and they are progressively dehydrated.

It has been possible to establish, in particularly favourable material, that each chromosome is made up of two separate strands which are called chromatids and are closely associated throughout their length without fusing. The shortening of the prophase chromosome is actually due to a progressive coiling of the single chromatids which become also twisted around each other. Recent research indicate that in some instances each prophase chromosome is actually composed of four separate strands or chromonemata, two for each chromatid.

The two pairs of homologous chromosomes are outlined in Fig. 1.1 where a telocentric and a metacentric chromosome are represented twice. The shape and size of the chromosomes are already well characterized toward the end of prophase.

The end of prophase is marked by the disappearance of the nucleoli and of the nuclear membrane.

Prophase is also characterized by the division of the centriole into two daughter centrioli that gradually separate while a system of asters develops above each of them. They migrate along the nuclear membrane describing a semicircular path toward the poles (Fig. 1.1 b,c) and, when they have reached antipodal position and the nuclear membrane is dissolved, a bundle of delicate filaments, or spindle fibres, appears between the two asters. Only the fibres connecting chromosomes with asters have been outlined in Fig. 1.1 d,e,f. This is the way the achromatic spindle seems to be formed during the cell divisions of most Metazoa.

When the nuclear membrane has disintegrated the chromosomes move in the centre of the cell toward the plane of the equator of the spindle where either they become irregularly arranged (as in most plant cells) or they arrange themselves radially at the periphery of the spindle (as in animal cells) and form the equatorial plate.

Mitosis has thus reached the *metaphase* stage and the chromosomes are attached to the spindle fibres by means of the centromeres that mark their primary constrictions.

The division of the centromere, to which the two chromatids were united, characterizes the beginning of the *anaphase* stage (Fig. 1.1 e,f). The chromatids begin thus to move toward the poles forming the daughter chromosomes.

In the meantime the spindle fibres in the zone between the two groups of chromosomes, which are named the interzonal fibres, stretch and an elongation of the spindle results in some organisms.

Telophase (Fig. 1.1 g,h) begins where the movements of the two daughter groups come to an end in the polar regions. A regrouping of the chromosomes into a nuclear structure takes place and their spiralization appears in favourable preparations at this stage within the newly formed nuclear membranes.

The process of segmentation and separation of the cytoplasm is obtained in some animal cells through a constriction in the equatorial region which is gradually accentuated until two separate cells are formed. In cells possessing rigid membranes a cell plate is formed between the two daughter cells while the fibres of the spindle vanish gradually from the central to the marginal zone.

Cell division may differ considerably from this generalized scheme, as for instance in some Protozoa where the entire mitotic cycle takes place inside the nuclear membrane.

Detailed comparative studies have nevertheless demonstrated that, as a result of mitosis, chromosomes in equal number, form and structure are distributed from mother to the two daughter cells. In the course of the analytic work that lead to this conclusion the morphology of the chromosomes has been very carefully investigated and the already mentioned morphological types of chromosomes have been described.

The general principle of the individuality and constancy in number of the chromosome set within each species was thus established. When the chromosomal theory of inheritance was demonstrated (Morgan, Bridges) it was also realized that the precision of the mitotic cycle implies the exact distribution to the daughter cells of the genes, which are located in the chromosomes. Deviations from the normal course of the mitotic processes may have important consequences in sex determination.

2. *Mitosis and Sex Determination*

Cleveland (1949) described in *Trichonympha*, a hypermastigote flagellate that lives in the intestine of the American cockroach *Cryptocercus punctulatus*, a highly aberrant type of mitosis, which has the character of a sex differentiating division. Twenty-four chromosomes appear during the prophase of the division that gives rise to cells called gametes. The chromosomes are early divided into two series of chromatids which are distinct through their colorability with haematoxylin: the chromatids that will reach the male gametes are more colourable than the chromatids that go to the female gametes and their division in the two daughter cells is not casual.

According to Cleveland's description, which is accompanied by excellent drawings and photographs, chromatids appear to unite in groups of four to form pseudotetrads and two chains of six such groups appear in late prophase and in metaphase (Fig. 1.2). The stronger coloured chromosomes go after that to the cell that will differentiate as a male gamete (where the male pronucleus is originated) and the less coloured to the future female gamete.

The resemblance of the rings with those which are formed by translocation heterozygotes is evident. There is, however, no suggestion of the possibility that a meiotic stage may take place during gamete formation in *Trichonympha*. As a further comment to this exceptional type of division it can be added that the differentiation into male and female chromosome sets may be regarded as the consequence of a segregation of sex genes which has actually taken place at meiosis.

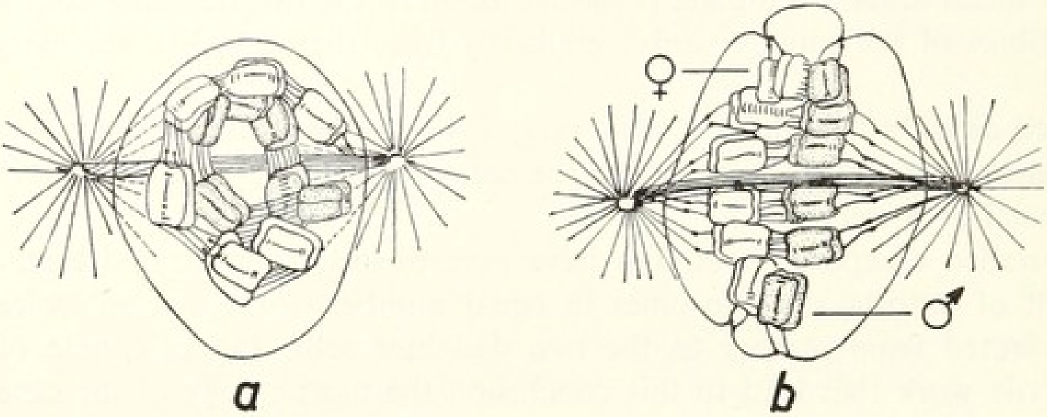

FIG. 1.2. Differential mitosis in *Trichonympha*. Dark and light groups of four chromatids have joined to form two rings in prophase (a) which are going to apposite poles (b) to form the nuclei of the male and female gamete (Cleveland, 1949).

More recent work by Cleveland (1956) showed that oxygen concentrations of 70–80 per cent of an atmosphere destroy all chromosomes of *Trichonympha* during the early stages of gametogenesis. Two anucleate gametes are produced after this treatment and they make nevertheless some progress in the cytoplasmic differentiation toward normal male and female cells.

Puzzling as they may appear, it must be noticed that the results on sex differentiation in *Trichonympha* resemble the well-known differentiation of migrating "male" pronuclei and stationary "female" pronuclei which result from the mitotic division of the surviving haploid nucleus during conjugation of Ciliates (see p. 33). Gametic production is accompanied in *Trichonympha* by a differentiating process which appears evident also in the chromosomes. The mitotic division preceding gamete formation in Ciliates may also have the character of a sex differentiating process without being detectable as such through a peculiar behaviour of the chromosomes.

Despite the outstanding results obtained by some authors, the cytological processes of Protozoa appear at present too little known to provide a firm basis for the explanation of sex differentiation which follows apparently normal mitotic divisions.

Differential mitotic divisions, which are characterized by the elimination of chromosomes at different embryological stages, determine sexual differentiation in species of two families of Diptera, the Sciaridae and the Cecydomyidae (see p. 85). A similar process takes place in the nematode worm *Angiostomum* and is sometimes observed in animal groups where amphigonic alternate with parthenogenetic phases (see Chapter 11).

Elimination processes in mitosis have thus become a feature of the normal life cycle in some Metazoa but disturbances occurring in the course of mitotic divisions at more or less early life stages produce the exceptional phenomenon of gynandromorphism (see p. 80) which has had much importance in determining the process of sex determination.

It was noticed that mitosis insures that every kind of self reproducing elements of the nucleus become identically distributed in the daughter cells. For this reason alterations in this process often cause important disturbances in the inheritance of sex.

3. *Meiosis, Segregation and Recombination*

Meiosis halves the number of chromosomes and it takes place during gamete formation in most animals and during spore formation in most plants. It essentially consists of two divisions of the nuclei that follow each other rapidly while a single chromosome division takes place. Two meiotic or maturation divisions must therefore be considered. The first division has been designated the reductional division because it reduces the chromosome number and the second division is called equational and is apparently mitotic.

The *prophase* of the first division is very long and involves very significant modifications. It is simply called the meiotic prophase and it has been divided into five stages.

Leptotene stage (Fig. 1.3a). In this early prophase stage the filaments of the diploid set of chromosomes appear very long and slender and show a series of chromomeres which look like the beads of a rosary. In this stage the chromosomes may be arranged at random or they may lie with a definite orientation and be polarized with their ends at a side of the nucleus and the remaining portions spraying out in the interior of the nuclear membrane. This arrangement is called synizesis and it may be observed also in the following stages.

Zygotene stage. The pairing of the homologous chromosomes, which are of paternal and maternal origin, takes place at this stage. This process of union, which is named synapsis, starts at different places along the chromosome filaments and it is completed at the pachytene stage.

Pachytene stage (Fig. 1.3b). The pairs of chromosomes, which form the so-called bivalent chromosomes, have become shorter and thicker and are present in half the initial number of chromosome filaments. Each homologous chromosome of the new units that have thus developed, splits at this stage into two daughter chromatids and bivalents are said to form tetrads, being apparently composed of four chromatids.

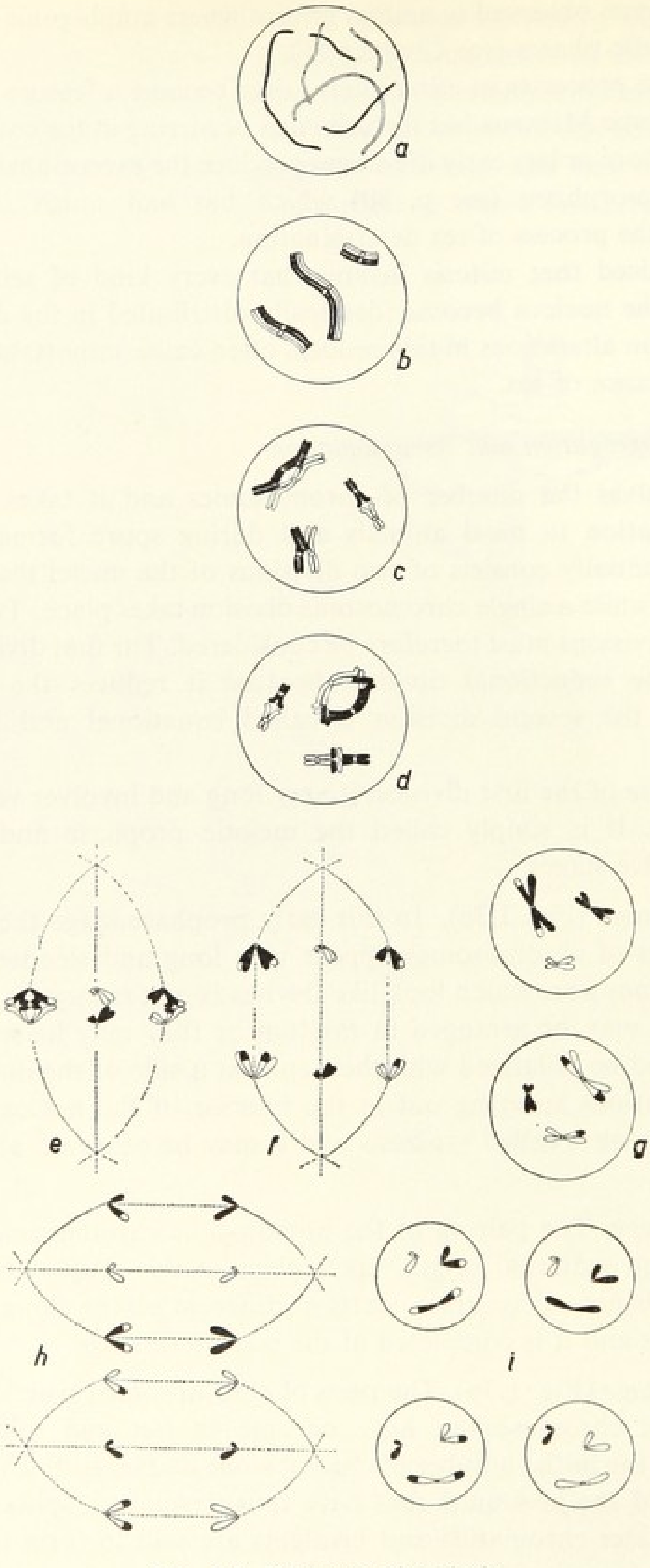

FIG. 1.3. Diagram of meiosis.

The longitudinal cleavage of each homologous chromosome is accompanied at pachytene by breaks in two homologous chromatids, which take place at the same level. An interchange of both segments of the chromatids takes place immediately and mixed chromatids are formed after the fusion between homologous chromatids. This process implies an interchange of genes from homologous chromosomes, that is from chromosomes of paternal and maternal origin, which have been represented in the diagram as solid and outlined respectively.

Diplotene stage (Fig. 1.3c). The paired chromosomes start to separate at this stage by apparently repelling each other. The separation, however, is generally not complete for the homologous chromosomes are held together at the points where two of the chromatids have crossed over. Each point of contact, corresponding to a point of interchange, is called a chiasma and at least one chiasma is formed for each bivalent. Some chiasmata are interstitial, between the ends of the chromosomes, and some are terminal. When a single chiasma is present the bivalent takes the form of a cross, when two or more chiasmata are present a loop, a double loop or a series of loops is formed.

During this stage the chiasmata are progressively displaced along the chromosome from the centromere towards the ends, a movement that has been called terminalization and is completed at metaphase.

Diakinesis. The processes of the previous stage are accentuated: chromosomes are very contracted, the number of interstitial chiasmata decreases through terminalization and the homologues appear joined to each other at their ends. The nucleolus is detached from its chromosome or has completely disappeared. This stage comes to an end with the disappearance of the nuclear membrane.

Metaphase I (Fig. 1.3d). In this stage, which takes place soon after diakinesis, the bivalent chromosomes orient themselves in the equatorial plane, and the centromeres of the two homologous members of each bivalent are directed toward opposite poles. The formation of the spindles takes place in the meantime as in mitosis.

Anaphase I (Fig. 1.3e). In this stage the chromosomes of each bivalent move from the metaphase plate to their respective poles while the chiasmata are dissolved. A segregation of whole chromosomes instead of chromatids takes place in this way because each centromere remains undivided and the number of chromosomes is halved. On the other hand the homologous chromosomes have now a different composition from that of the originals as a consequence of the crossing over between chromatids. After *telophase* I (Fig. 1.3f) and *interphase*, which have the same character as the corresponding mitotic phases, a short *prophase* II takes place.

Metaphase II. Is the most significant phase of the II meiotic division, the chromosomes appear in reduced number with the chromatids widely separated from each other. *Anaphase* and *telophase* II (Fig. 1.3 g,h) have a mitotic character.

The above succession of nuclear events in meiosis takes place in most sexual organisms with only minor variations and important deviations are mostly observed in meiotic processes of parthenogenetic species (see Chapters 10 and 11.) It can be mentioned among the peculiarities of meiotic processes that no chiasmata are formed in the course of spermatogenesis in most Diptera although Cooper (1949) did observe chiasmata in male *Drosophila*.

Startling examples of one division meiosis have been described in *Saccinobaculus*, in *Oxymonas* and in *Notila*, which—like the above mentioned *Trichonympha*—are symbiont flagellates of *Cryptocercus*. Cleveland (1950) showed that diploid gametes* or conjugants of *Notila* undergo plasmogamy without fusion of the nuclei and remain in this condition for 7–8 days. Each nucleus undergoes then a single clear division which is regarded as meiotic since the chromosome number is halved. The subsequent fusion of haploid "male" and "female" nuclei develop diploid nuclei and cells that start reproducing by mitosis until the next molt of the host induces (see p. 18) sexual reproduction. Further caryological investigation will be needed in order to establish how the "one division meiosis" of symbiont flagellates fits in the present general interpretation of the meiotic process.

Although there is reason to expect that such abnormal sequences of meiosis are more widespread than was supposed a few years ago, we can nevertheless regard the following consequences of meiosis as firmly established:

1. The halving of the number of chromosomes so that a haploid set is obtained, where each chromosome is represented only once.
2. The separation of the four chromatid strands and their final segregation as chromosomes within four cells so that the germ cells are pure for either the paternal or the maternal alleles.
3. The crossing over between chromatids from homologous chromosomes, which implies recombination of linked genes in the progeny of heterozygotes.

The extreme variability which is shown by different organisms and sexes in the destiny and separation of the four products of the meiotic divisions will be analysed later.

4. *Meiosis and Sex Determination*

The behaviour of the so-called sex or X and Y chromosomes, which are distributed to one and not to another of the products of meiosis (McClung, 1902; Wilson, 1906), can be regarded as a deviation from the standard meiotic process although it has become established in quite a few unisexual

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* Cleveland's terminology is given here.

organisms in the course of evolution. Each of the sex chromosomes is subdivided into the differential segment, where crossing over is suppressed, and into the pairing segment which is homologous in X and in Y (Winge, 1923a; Darlington, 1931). Such chromosomes often remain condensed during the resting phase and appear thicker or thinner than the other chromosomes in certain stages of the nuclear cycle. They are said therefore to show heteropycnosis and to be composed of heterochromatin at least in some regions. The existence of the sex chromosomes with their peculiar behaviour implies the formation of two types of gametes or spores and results in the mechanism of sex digamety which has been regarded until recently as the general model of genetic sex determination.

Recent comparative research tends, however, to consider this mechanism as representing a highly specialized device which is limited to a minority of organisms like nematodes, insects, some arthropods and higher vertebrates among animals. Sex chromosomes have been observed in a few angiosperms and in some Bryophytes among plants.

Non disjunction and loss of sex chromosomes during meiosis give rise to some of the most important anomalies in sex determination. Intersexes in *Drosophila* and gynandromorphism or sex mosaicism in man are often caused by such errors in meiosis.

The random recombination of multiple sexual factors during the meiotic process appears now to be at the basis of sex determining mechanisms in a number of unisexual (Kosswig, 1939b) and hermaphrodite organisms (Bacci, 1955), as it will be shown in later chapters.

5. *Fertilization, Diploidy and Haploidy*

Meiosis is necessarily correlated in the sexual cycle with the opposite and complementary process of fertilization which consist in the fusion of two gametes of opposite sex (♂ and ♀, + and —).

Caryogamy or the fusion of nuclei of gametes is the culminating part of fertilization. By this process the homologous chromosomes that were separated in the course of meiosis are brought together again.

Fertilization is carried on in different species by mechanisms that are far more varied than those observed in meiosis. Some of them will be illustrated later in the course of an analysis of different sexual cycles, for the present it is sufficient to describe the process of fertilization as it takes place in the hookworm *Ascaris megalocephala univalens* where it was studied by van Beneden (1876) shortly after O. Hertwig first described fertilization in an echinoid egg (1875). The male gamete or sperm of *Ascaris* adheres to the surface of the egg while the egg nucleus is still in the prophase stage (Fig. 1.4a). Immediately after the sperm, which is devoid of the tail, has penetrated the egg the first maturation division takes place with the division of the first polar body from the first oocyte while the sperm nucleus changes its original form to that of a male pronucleus (Fig. 1.4c). The meiotic division of the egg is completed with the formation of the second polar body and in the egg remain two chromosomes (Fig. 1.4d) which form the female pronucleus. The male pronucleus assumes then a prophase aspect and two distinct chromosomes appear inside the nuclear membranes both of the male and of the female pronucleus (Fig. 1.4e). In the meantime two centrioles appear at the side of the nuclei and soon the nuclear membranes dissolve and caryogamy is accomplished while the mitotic metaphase of the first division of the new organism is initiated. The classical example of fertilization in *Ascaris* makes clear that the main result consists in the formation of a cell, the zygote, where two pairs of homologous chromosomes are present after the union of the two sets of chromosomes both from the sperm and the egg nucleus.

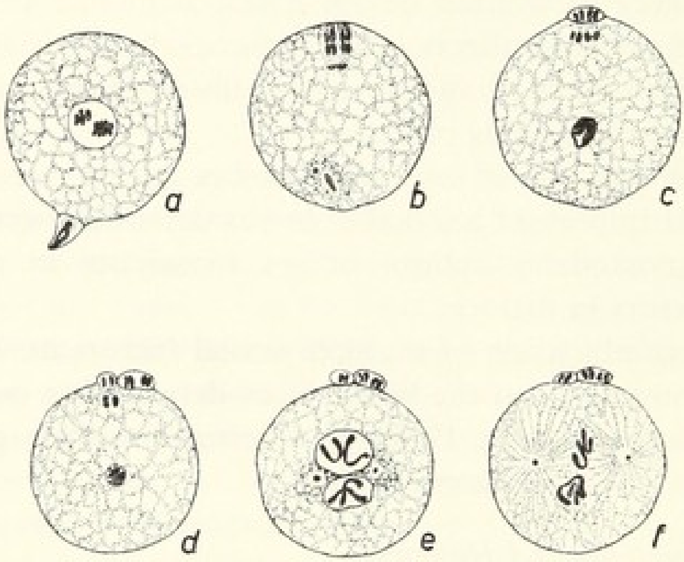

FIG. 1.4. Fertilization and caryogamy of *Ascaris megalocephala*. The two meiotic divisions of the egg nucleus are taking place (a to d) after sperm penetration (after van Beneden).

Fertilization restores the diploid condition by uniting paternal and maternal homologous chromosomes carried by the haploid gamete cells which result from the meiotic process either directly or after a series of mitotic divisions. In this way *n* pairs of homologous chromosomes are present in the zygote whose diploid number of chromosomes is indicated by 2*n* and only *n* different chromosome are present in the cells or organisms that are derived from the meiotic process.

As a consequence the sexual reproductive cycle consists in the alternation of haploidy and diploidy due to the sequence of caryogamy and meiosis with mitosis assuring the accurate transmission of the heredity material.

6. *Amphimixis and Automixis*

Fertilization generally takes place between cells that have been produced by different individuals. In such instances it is called amphimixis, which is certainly prevalent also among hermaphrodite organisms.

Self fertilization, which is a type of automixis, is on the contrary rather uncommon and it is prevented in many instances by a number of genetic and physiological devices. It is nevertheless widespread in a number of organisms, for instance in Rhizopoda, where the case of *Actinophrys sol* represents an outstanding example (Belar, 1922). Examples of self fertilizing Metazoa are frequently being discovered (Cognetti, 1956a; Delavault, 1958; Parenti, 1960) and several normally autogamous plants are also well known. Automictic parthenogenesis (see page 216) is also essentially a kind of self fertilization. It is possible therefore that the prevalence of amphimixis over automixis has been overestimated under the influence of theoretical considerations.

The most important deviation from the normal sequence of events in fertilization is perhaps shown by certain Fungi where two haploid nuclei from different mycelia migrate in the same cell without undergoing caryogamy and divide several times in the same mycelium by conjugated mitotic division until caryogamy takes place. Thus plasmogamy and caryogamy appear as quite distinct phases in the fertilization process.

Other minor variations in fertilization are represented by the fact that gametes may or may not show differences in size or may be represented by nuclei that are not surrounded by a cellular body having a definite boundary.

Reproduction and Cellular Differentiation

The alternation of nuclear diploid and haploid phases in sexually reproducing organisms is accompanied by a series of cytoplasmic modifications of the reproductive cells which is more or less evident in different organisms. This is true not only for most of the cellular elements that take part in fertilization processes but also for most of the reproductive elements that give rise to new individuals in the absence of fertilization and are said therefore to reproduce agamically or asexually.

1. Diffusion and Types of Asexual Reproduction

Organisms that reproduce in the absence of any sexual process are not uncommon although recent investigation has shown the existence of a sexual cycle in many groups where sexual processes were unknown. Neither fertilization nor meiosis have ever been observed among Chloromonadina, Silicoflagellates, Cryptomonadina and in most dinoflagellates. Sexual processes are also unknown in the successful and widespread genus *Chlorella* and in most species of *Chlamydomonas*. Most zooflagellates and amoebina seem also to reproduce only asexually without any trace of sexuality. Very few asexual species are known, however, among higher plants and animals.

Asexually reproducing individuals are also known to occur in regular or irregular alternation with sexually reproducing individuals in many species.

Exclusively asexual reproduction was formerly considered as primitive in consideration of the complexity of the sexual cycle and of the low taxonomic position of most asexual organisms. Now that the existence of quasi sexual processes has been demonstrated in various strains of bacteria (see Chapter 3) there is a tendency to attach a fundamental importance to the sex cycle and to assume that sex has been secondarily lost in asexually reproducing species. As a matter of fact no definite position can be held at present in this respect, considering how little is known about the life cycles of the so called primitive organisms. Only in metazoa and in angiosperms can agamic reproduction be regarded as the result of a secondary evolution.

Asexual reproduction may take place with the development of a new individual from a single cell, and it has been called agamogony (Hartmann, 1904), or from a group of cells. It is called vegetative reproduction *sensu stricto*. This distinction is based upon the germ plasma theory of Weismann (1886a) as in agamogony the single asexual cells either belong to unicellular organisms, where a somatic and a germinal line cannot be distinguished, or they belong to the germ line. In vegetative reproduction the new organism develops from either undifferentiated or dedifferentiated cells, that in most cases cannot be said to belong to a specialized germ line. Although the germ plasma theory has lost much of its former interest, the concept is useful because it is a well established fact that agamogony takes place with peculiar and uniform characters in the most varied organisms while processes of vegetative reproduction differ very much in the different organisms where they appear as *ex novo* adaptation.

Agamic reproduction in both its aspect of agamogony and vegetative reproduction may represent the only reproductive process of a species or it may take place within the sexual cycle of an organism as an essential or inessential part of its life cycle.

2. Agamogony

Agamogony of the unicellular organisms, where a germ line is not separated, takes place essentially in three ways: (1) equal binary fission as in some *Chlamydomonas* (Fig. 2.1), in *Amoeba* or in *Paramecium;* (2) budding, as in Suctoria; (3) multiple fission, in which the nucleus divides several times before the cytoplasm falls into as many parts as there are nuclei. Multiple fission is well known especially in Sporozoa (Fig. 2.2). Agamogony is the only way of reproduction in some Amoebina and it represents simply a step of the complicated sexual cycle of the Sporozoa.

Highly differentiated processes of agamogony lead to the production of specialized germinal cells that are called spores and develop into a new organism without fertilization. They may be obtained as the immediate result of meiosis as in *Ectocarpus* (Fig. 2.3), in mosses (Fig. 2.4), in angiosperms or in Basidiomycetes (Fig. 2.9) and give rise to haploid organisms which are constant elements of the sexual cycles. They can also be produced mitotically by the haploid or by the diploid organism and give rise to other organisms having the same chromosome number of the parent individuals.

The term spore may thus seem to be too comprehensive but more accurate terminologies are based on different and often contrasting principles that may lead to extreme confusion.

Spores may show quite different shapes which are clearly related with the environment where they develop. Some of them are able to move by means of flagella, as in most algae and in Phycomycetes, or by pseudopodia, as in Foraminifera, and they are called zoospores or planospores. Nonmotile and mononucleate spores, aplanospores, are formed in *Microspora*, and occasionally in *Ulothrix* among Chlorophyceae. Ascospores, basidiospores and conidia produced by Fungi are among the best-known examples of nonmotile spores.

Spores in angiosperms are developed as the direct result of meiosis and, like gametes, they are typically formed in groups of four. A differentiation exists among angiosperms between the male producing microspores and the female producing megaspores and this means that a morphological predetermination is established in asexual reproductive cells. The diploid megasporocyte goes through meiosis and produces a tetrad of four haploid cells but only one becomes the functional megaspore, a condition that closely resembles that of egg formation in metazoa (see p. 18). Thus heterospory is represented in higher plants beginning with some of the pteridophytes. Lower plants are on the contrary isosporic although a genetic sex predetermination has been demonstrated in some instance.

Spores are often produced and protected by special organs that are called sporangia and have the most varied structure in relation to the way of dispersal and the life cycle.

3. *Vegetative Reproduction* sensu stricto *and Apomixis*

Vegetative reproduction *sensu stricto*, which is characterized by development from cellular complexes, is especially important among higher plants which develop special organs of vegetative reproduction, such as the tuberous swellings of the potato plants, the bulbs of onions and of hyacinth, the underground stems of many grasses and the runners or stolons of strawberries (Fig. 1.5).

Upon the ability of parts of many plants to produce adventitious roots is also based the widespread practice of taking cuttings, which makes possible not only a rapid production of new individuals, but also assures the constancy of the morphological and functional characteristics of the parent plants as it is based upon the precision of the mitotic division. Some species of angiosperms are known to reproduce only by vegetative propagation.

Gametes are often replaced in plants by unspecialized cells which do not fuse and apogamy takes place, a process that must be distinguished from parthenogenesis because the embryo does not develop in apogamy from the egg cell. Somatic cells are also involved in apospory where a diploid embryo sac is formed directly by a series of cell divisions starting from elements of nucellar or integumental origin. Such processes are classified under the non-committing term of apomixis indicating the external form of sexual reproduction where gamete formation and meiosis are usually omitted.

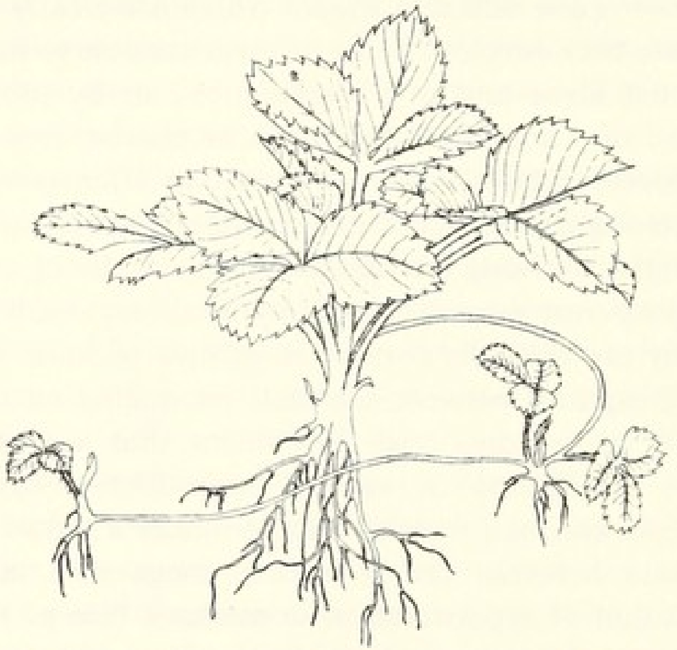

FIG. 1.5. A strawberry reproducing vegetatively by stolons.

Exclusively vegetative reproduction is rather rare among metazoa where indefinite asexual reproduction has been observed in *Hydra fusca* (Brien and Reniers Decoen, 1949), in the polychaete annelid *Ctenodrilus monostylos* that has reproduced asexually in aquarium for 60 yr (Korschelt, 1942), and in a number of limicolous oligochaeta of the genera *Nais* and *Aelosoma* (Maupas, 1919).

Processes of vegetative reproduction are closely allied with those of regeneration (Korschelt, 1927, Vorontsova and Liosner, 1960) and are made possible by the existence of what has been named an embryonic reservoir (Brien, 1931) embedded in the soma. It can be accelerated, like regeneration, by a number of external influences but it takes place according to processes that follow well fixed rules in the different species (Herlant Meewis, 1958): the continuity of characters assured by this type of reproduction is based upon the constancy of its methods.

The alternation of vegetative with sexual reproduction will be discussed in Chapter 2.

4. *Gametes and Gametogenesis*

No apparent differences between asexual cells and gametes, that are specialized for fertilization, are observed in some species of *Chlamydomonas*.

Very small differences can also be observed in the chlorophycean genus *Chlorogonium*. In *Chladospora*, another chlorophycean, gamete formation is similar to zoospore formation, but gametes are biflagellate and zoospores are quadriflagellate. Differences between gametes and agametes are definitely established in higher organisms.

In some lower organisms fertilization takes place between gametes of identical size and structure and no morphological distinctions are possible between male and female gametes in such cases of isogamy. In anisogamy both gametes are flagellated, but one of them is regularly larger than the other. Oogamy is characterized by the differentiation of a small flagellate male gamete (antherozoid or sperm) and of a large non-flagellated female gamete (or egg). Although oogamy is the type of gamete differentiation peculiar to Metazoa and to highly evolved phyla of plants, it occurs also in genera where, as in *Chlamydomonas*, some species produce isogametes.

Male flagellated gametes are produced in Chlorophyceae, Chrysophyceae, in Phaeophyceae as well as in Bryophyta and in Pteridophyta. Male gametangia, called antheridia, produce such male gametes or antherozoids and female gametangia, oogonia or ascogonia, produce the nonmotile egg cell.

Unflagellated male gametes resembling fungal conidia are formed in Ascomycetes. They are named spermatia or microconidia and are produced either in flask-shaped cavities or upon special hyphae where they develop singly or in clusters. The ascogonium of Ascomycetes is either unicellular or multicellular and its apex is usually prolonged into a trichogyne which grows toward the spermatia that lodge in the vicinity.

The plant egg attains its greatest size in gymnosperms but never reaches the dimensions or complexity of structure shown by some animal ova. Plastids are frequently present in the eggs of higher plants and they appear in the gametes of both sexes in most algae. In angiosperms the egg nucleus is formed in the embryo sac and only after fertilization it gathers a spheroidal cytoplasm showing a definite boundary (Fig. 2.5).

Non-flagellate sperm cells are represented in angiosperms by the two generative nuclei borne within the growing pollen tube (Fig. 2.5). The sperm of the higher gymnosperms is a complete cell surrounded by a definite boundary and it shows some of the characters of the antherozoids of bryophytes and pteridophytes.

Gametes are produced in many Protozoa, by a common mother cell that is named gamont. It must also be remarked that unicellular haploid organisms are often both sexual organisms and gametes. Fertilization is frequently preceded in Protozoa by the union of two gamonts and a process of gamontogamy takes place.

Maturation and differentiation of gametes in Metazoa generally take place in special organs called gonads that may be distinguished in an ovary and in a testis or may be fused in an ovotestis. The meiotic division form part of the process of gamete formation and, since gametes result from the two successive meiotic divisions, they are typically formed in quadruple groups. Germinal cells in the gonads are initially undifferentiated, that is they are similar in both sexes, and are then named protogonia (Beccari, 1924). Initially they divide mitotically to form gonia that are distinguished into spermatogonia and oogonia. The former will develop male and the latter female gametes. Primary spermatogonia and oogonia develop secondary spermatogonia and oogonia during the so-called division period. When mitotic divisions have stopped a period of intense growth takes place and spermatogonia are transformed into primary spermatocytes, oogonia into primary oocytes. Primary oocytes are much bigger than spermatocytes.

The first meiotic division develops in spermatogenesis two secondary spermatocytes and each of them divides into two spermatids with the second meiotic division. In this way the maturation period, which started with synapsis in primary spermatocytes, is over and spermiogenesis begin with a complicated series of modifications which lead to the formation of the ripe sperms which are often flagellate.

The course of ovogenesis is quite different as a consequence of the tremendous growth of the primary oocytes (Fig. 1.6). Chromosomes are well distinguishable up to diplotene stage, after which they almost disappear in the nuclear sap and may take on the aspect of lamp-brush chromosomes. The maximum size of the oocyte is usually reached at this stage (Fig. 1.6e). Then diakinetic chromosomes reappear and the first meiotic metaphase takes place immediately. Two cells of very unequal size are originated after the first meiotic division: a secondary oocyte and a small first polar body (Fig. 1.6h) that usually does not undergo the second meiotic division. The second meiotic division of the secondary oocyte forms a ripe egg and the second polar body (Fig. 1.6i). If the first polar body has divided, the maturation division from the first oocyte has developed one functional germ cell, the egg, and three abortive germ cells, the polar bodies or polocytes, which are homologous to the four sperms formed as a result of the meiotic divisions in the course of spermatogenesis.

Thus the division of labour between the gametes of the two sexes reaches among Metazoa its highest expression with the accumulation of deutoplasm in the growth of the oocyte and with the complicated spermiogenic processes that confer the sperm its extreme mobility.

5. The Induction of Gametogenesis

Environmental factors decide in a number of organisms whether germ cells develop as agametes or differentiate into gametes, that is whether asexual or sexual reproduction will take place.

It appears that in *Chlamydomonas* all the small cells recently liberated

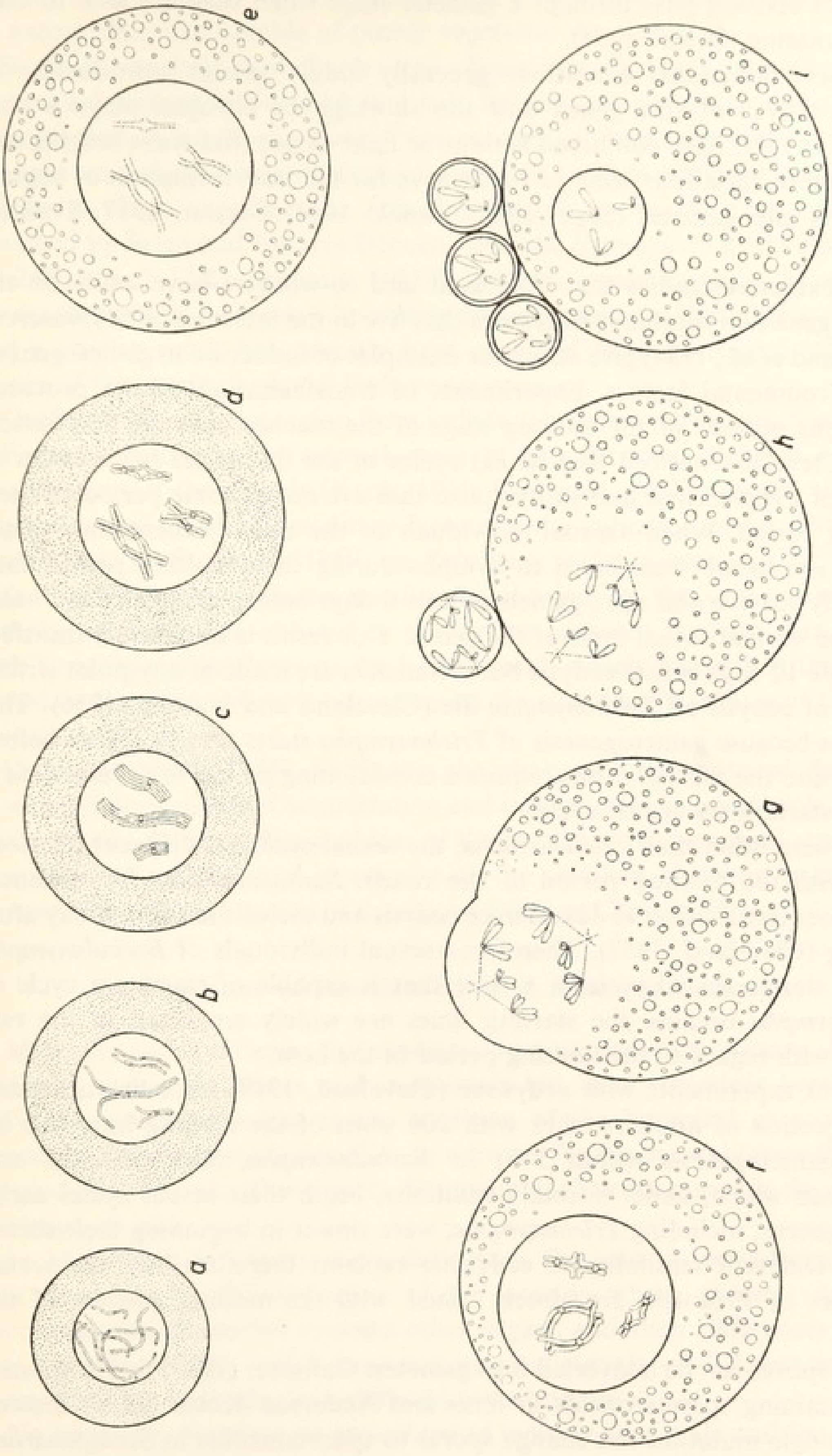

FIG. 1.6. Ovogenesis in Metazoa: only two polar bodies are generally formed instead of three (i), the maximum size is reached at formation of lamp-brush chromosomes (e).

after cell division pass through a gametic stage when they are able to take part in mating (Lewin, 1954).

Adverse nutritional conditions generally induce sexual reproduction in representatives of this genus and the shortage in nitrogen plays a very important role. Also combined actions of light of peculiar wave lengths and temperature variations seem to be decisive for the transformation of vegetative cells into gametes (Sager and Granick, 1954; Forster, 1957; Trainor, 1958).

The extensive studies that Cleveland and co-workers have made on the several genera of symbiont flagellates that live in the intestine of *Cryptocercus* (Cleveland *et al.*, 1934) give also clear examples of induction to gametogenesis by environmental factors. Experiments of transfaunation of the protozoa in various periods of the molting stage of the roaches gave the first indications. Cleveland noticed that sexual cycles of the flagellates never occur in adults of *Cryptocercus* and in nymphae that are not at some period of their molting period. When asexual individuals of the already mentioned genus *Trichonympha* are transferred to nymphs during their molting period they generally survive and start gametogenesis 6 days before ecdysis of the hosts as in the normal sexual cycle of the genus. This result is obtained if transfers are made 12 days before ecdysis but if transfers are made at any point within 9 days of ecdysis all *Trichonympha* die (Cleveland and Nutting, 1956). This happens because gametogenesis of *Trichonympha* starts always 6 days before ecdysis and the asexual forms require a conditioning period of at least 3 days before starting the sexual cycle.

In genera other than *Trichonympha*, the sexual cycle has different relationships with the molting period of the roach. *Barbulanympha*, for instance, undergoes fertilization 45 days before ecdysis and meiosis in the first day after molting (Cleveland, 1957). Therefore asexual individuals of *Barbulanympha* cannot start a sexual cycle in a host that is capable of starting a cycle in *Trichonympha* because the starting times are widely separated in the two genera with regard to the molting period of the host.

Recent experiments with ecdysone (Cleveland, 1959) have demonstrated that injection of adult roaches with 200 units of the molting hormone induces gametogenesis within 3 hr in *Barbulanympha*, *Saccinobaculus* and *Oxymonas* which, under normal conditions, begin their sexual cycles early. Other genera, including *Trichonympha*, were slower in beginning their sexual cycles. Different sensibility to ecdysone explains therefore how the sexual processes of Protozoa are closely tuned with the molting process of the roach.

Also spores can be converted into gametes. Gairdner (1933) found sporangia containing spermatozoids in ferns and Anderson–Kotto (1936) showed that a single mutation can change spores to spermatozoids in *Scolopendrium vulgare*. Also the reverse process of transformation of gametes into agametes can take place. Dodge (1932) demonstrated that microconidia of *Neurospora*, an ascomycete, are capable of purely vegetative development.

Such observations show that a close affinity actually exists between asexual and sexual germ cells although they help little in understanding evolutionary changes that in nature may lead from asexual to sexual reproduction. Such changes can only be obtained through a series of small co-ordinated mutations.

Passage from vegetative to sexual reproduction in Metazoa has been obtained by Brien and Reniers Decoen (1949) in *Hydra fusca* by cooling from 15–24°C to 7–12°C. Loomis (1957) induced gametogenesis in asexual *Hydra littoralis* by increasing the carbon dioxide tension of the culture medium and a similar result has recently been obtained by Bravermann (1962) in clones of the marine hydroid *Podocoryne carnea*.

Grafts of the cephalic region from individuals belonging to sexual races on individuals belonging to asexual races of the flatworm *Dugesia* induced development of the gonads (Kenk, 1941; Okugawa, 1957) showing thus the importance of racial or hereditary factors in the induction of sexuality. The experiments by Stunkard (1959) on *Polystoma stellai*, a monogenetic trematode, that lives on *Hyla septentrionalis*, showed on the other hand that gametogenesis can be induced in the unripe parasites through stimulation with hypophyseal implants on the host, a result closely resembling those obtained on the action of molt hormones on the symbiont flagellates of *Cryptocercus*.

Durchon showed (1958, 1959) that the pharyngeal proventricule function as an inhibitory centre of sexualization and stolonization in polychaete worms of the family Syllinae. The problem of the factors inducing sexual reproduction becomes at this point identical with the problem of the embryological factors inducing maturation of the sexual cells (see Chapter 9).

Nomenclature of Sex Conditions in Plants and Animals

Maleness and femaleness are expressed in a variety of conditions that are basically similar in plants and in animals but have sometime been given different names according to the tradition of the botanical or zoological literature. It is important therefore to account for the general terms that will be used in this book, after a short discussion of their meanings in the current literature on sex.

In Metazoa it is important to distinguish the primary sexual characters that concern the differentiation of the gonads where gametes develop. Secondary sex characters concern other organs and their differences constitute sex dimorphism which may concern individuals of different sexes or hermaphrodite individuals in different sexual phases.

Unisexuality, dioecism or gonochorism indicate that male and female sex organs occur in different individuals, plants or animals. The three terms are almost perfectly equivalent although the last one is mostly used by zoologists.

The term hermaphroditism is employed for all the animals where male and female gametes reach maturation at the same or at different period of the life cycle (contemporary or consecutive hermaphroditism) and therefore the term includes also individuals with separated female and male gonads as well as individuals with male and female gametes developing in the same gonad or ovotestis. Plants are called hermaphroditic when the two sexes are found in the same flower, and they are generally called monoecious when male and female gametes mature in different flowers on the same plant. The term hermaphroditism will be used in this book in the widest implication of its meaning and practically coincides with that of bisexuality which is preferred by botanists.

It must also be pointed out that in cycles where parthenogenesis alternates with amphigony (see p. 42) the parthenogenetic generations are sometimes called unisexual because only the female sex is present in them and the amphigonic generations are called bisexual because both sexes are present. No confusion is possible, however, in such instances with gonochorism and hermaphroditism respectively.

Consecutive hermaphroditism may be either protandrous or protogynous, that is male or female gametes reach maturity first. Alternating hermaphroditism indicates the alternation of several opposite sex phases.

Strains of individuals that are developed through asexual reproduction from a single individual are called clones and they may include individuals of both sexes or only individuals belonging to a single sex. Thus monoecious or dioecious clones are distinguished.

Some animal populations have a majority of hermaphrodite individuals and a few pure male and female individuals (Bacci, 1947). Such populations or species are called unbalanced hermaphrodites. The contrary seems to happen more frequently among plant species and botanists call subdioecious the species where a few monoecious plants occur among a majority of unisexual plants. Male plants with female or hermaphrodite flowers are called subandroecious and female plants with occasional male or hermaphrodite flowers are usually called subgynoecious.

No special term has been proposed as yet to indicate hermaphrodite animal species where small complementary males occur.

The concept and term intersexuality have been introduced by Goldschmidt in the course of his pioneer work on *Lymantria* and have proved very helpful to indicate individuals which show intermediate sex conditions and even both prevalently male and female regions having the same genetic constitution. Intersexes are generally infertile or at most they produce ripe gametes of one sex only so that it is always possible to distinguish them from hermaphrodites which produce ripe gametes of both sexes. Intersexuality is often observed when individuals of different races or species are crossed and it occurs also in normal developmental stages of some organisms.

The so-called sex reversal may be a normal occurrence in the life cycle of some species. They can be regarded as consecutive hermaphrodites in which male and female gametes develop and reach maturity in quite distinct sex phases.

CHAPTER 2

REPRODUCTIVE CYCLES

MEIOSIS and fertilization divide the life cycles of sexual organisms into two parts, through the alternation of nuclear phases. After meiosis and before fertilization organisms are said to belong to the haplophase; after fertilization and before meiosis they belong to the diplophase. Asexual reproduction—as previously defined—may also take place either in the haplo, or in the diplophase or in both phases.

Meiosis takes place in some species immediately after the formation of the zygote, which is then the only diploid cell of the whole cycle. The organisms that undergo such zygotic reduction show the highest possible extension of the haplophase and therefore they are called haplonts.

Zygotic reduction takes place in Phytomonadina, in Coniugatae and in most Chlorophyceae. Phycomycetes, the most primitive among Fungi are also generally pure haplonts and among animals some Hypermastigina and apparently all Sporozoa undergo zygotic meiosis in their life cycles.

The intermediate reduction of haplo-diploid species (or haplo-diplonts) takes place after the diploid generation, represented among plants by the sporophyte, has developed from the zygote. Meiosis gives rise to spores or gonia and to a haploid generation (the gametophyte when plants are concerned) where gametes are formed. The relative extent of the haplo and diplophase varies in different groups and species.

Intermediate reduction is represented in some groups of Chlorophyceae and *Ulva* is a well-known example of equal development of the sporophytic and gametophytic phase. *Chaetomorpha* and *Cladophora* show also examples of this type of cycle. The brown algae *Ectocarpus* and *Dictyota* are haplo-diplonts as well as *Allomyces*, a member of the aquatic order of phycomycetes. It is well known that a progressive reduction of the gametophyte stage is observed in the life cycles of higher plants from Hepaticae to Angiospermae through mosses and ferns, a trend that is observed also in some genera of Phaeophyceae, the brown algae, and it appears to have some general evolutionary significance. Examples of haplo-diploid organisms among animals are found in the Foraminifera.

All pluricellular animals, some flagellates, some amoebae, Heliozoa and ciliates among the protozoa are pure diplonts, that is meiosis takes place at gamete formation (gametic reduction). A number of yeasts are also pure diplonts and diatoms, *Acetabularia* and *Fucus* among algae have evolved an exclusively diploid phase except in the gamete formation.

It has been remarked that in the case of diplonts and of haplonts chromosome reduction is not accompanied by a change in the type of reproduction while in haplo-diplonts the change from the diplo to the haplophase implies the passage from a sexually to an asexually reproducing generation. Thus a true alternation of generations takes place in haplo-diplonts because at least one asexual diploid generation and one sexual haploid generation alternate during the sexual cycle. Zygotes in the cycle of haplonts and gametes in the cycle of diplonts do not form a generation.

For this reason haplonts and diplonts are said to show homophasic, haplo-diplonts heterophasic alternation of generations (Hartmann, 1929). This distinction points out to the independence existing between the alternance in type of reproduction (metagenesis) and the alternance of nuclear phases that takes place in the different generations. It is important to note now that in the present survey, "generation" is defined as the phase in the life cycle of one species that extends from one to the immediately successive reproduction while in genetical research a generation corresponds to a whole sexual cycle. Thus in Mendel's experiments the different genotypes numbered at each generation result from processes that have taken place both in the sporophyte and in the gametophyte "generations".

The dicaryon, which is a peculiar nuclear association, must also be mentioned in a general survey of life cycles. Two sexual cells or organs, each containing one or more haploid nuclei fuse and the nuclei from the different strains remain associated in one or more pairs, each called a dicaryon, while retaining their individuality. In this way plasmogamy, or fusion of the cytoplasm, is separated from caryogamy in a kind of interrupted fertilization because the two members of each dicaryon may repeatedly undergo mitotic divisions (which are generally simultaneous) before performing caryogamy. Thus a dicaryotic phase may take place and haplo-dicaryotic and even dicaryotic cycles have been described in ascomycetes and in basidiomycetes.

Once these general principles have been established, only an analysis of single sexual cycles may give a concrete idea of the versatility of the sex cycle in animal and in plant species.

Haplonts

Organisms showing zygotic reduction belong to so-called primitive groups both among plants and animals and are adapted to the most varied habitats. Two examples are given from unicellular plants and animals.

1. Chlamydomonas

Species belonging to this genus of Phytomonadina live in marine and in freshwater and have been the subject of intensive research. In the simple form outlined in Fig. 2.1 the ripe zygote (Z) is spherical and is provided with a thick spiny wall. A meiotic division takes place (M) prior to germination (Moewus, 1936) and after a splitting of the zygote wall four haploid cells appear. They soon develop the two anterior flagella that are characteristic of the genus. In some species 8, 16 or even 32 cells may be formed within the zygote wall following two or three mitotic divisions. Each cell then increases in size and finally undergoes asexual reproduction by dividing longitudinally into two daughter cells that form walls before they free themselves from the parent cell wall. Four daughter cells are formed in asexual reproduction of most species. In the example given in the diagram of Fig. 2.1 cells belonging to the same clone become apposed at the anterior end and eventually fuse with each other without showing any morphological modification or differentiation while assuming the role of gametes. The quadriflagellate organism which is thus formed may remain motile for a long time until a spiny resting zygote is formed. The resting zygote represents thus the only diploid cell of this cycle and the haploid phase is represented in the diagram by a single line.

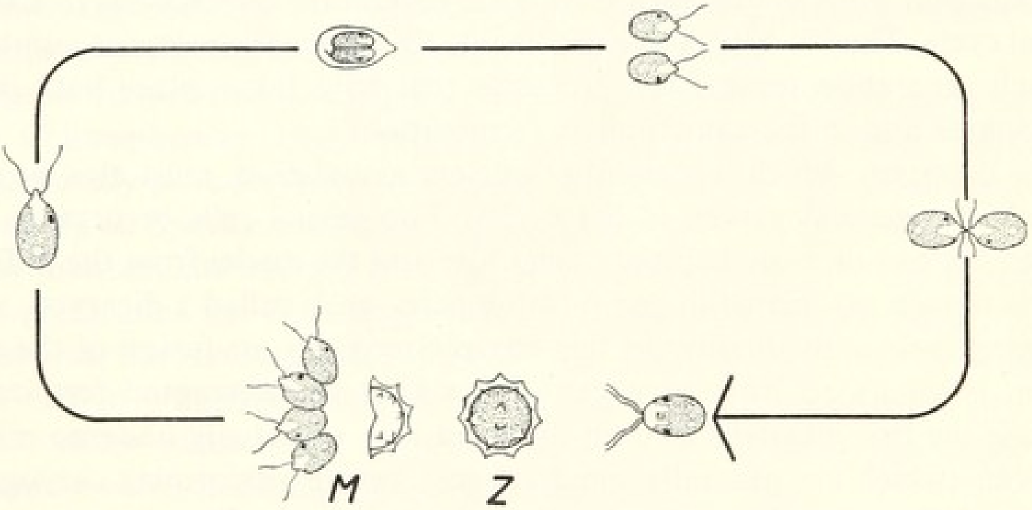

FIG. 2.1. The reproductive cycle in *Chlamydomonas* (a phytophlagellate). Z indicates the mature zygote, M the outcome of meiosis and the single line marks the persistence of the haploid phase.

The form considered here provides the simplest possible example of sex cycle: the gametes are isogamous because they show no morphological sex differentiation and the species is monoecious, homothallic or hermaphrodite since two gametes originating from a single cell (and thus belonging to the same clone) may unite to form a zygote. Dioecious species are also represented in the genus *Chlamydomonas*, as it will be shown later. Anisogamy is also shown by other *Chlamydomonas* species. It has been mentioned that transformation of asexual cells into gametes is favoured by external factors. A co-ordinating mechanism, which brings together different gametes and induces the so-called group formation is due to the action of chemicals, gamones that are produced by male and by female gametes (andro- and gynogamones) although it is still doubtful whether gamones directly determine the fusion of gametes. Förster, Wiese and Braunitzer (1956) demonstrated that the gynogamone of *Chlamydomonas eugametos*, a dioecious species, is a glycoprotein of spherical form approaching 10^8 in molecular weight.

2. *Aggregata eberthi*

This coccid, which lives as an endocellular parasite in the intestine of the cuttlefish *Sepia officinalis* and in the crab *Portunus depurator*, has been accurately studied by Dobell (1925) who established the haploid chromosome number of six for the whole cycle, with the exception of the zygote. This diploid stage (Fig. 2.2 Z) which lies in the intestinal wall of *Sepia*, undergoes meiotic reduction. A series of successive mitotic divisions lead to the formation of some small haploid sporoblasts which in turn are each transformed in sporocysts.

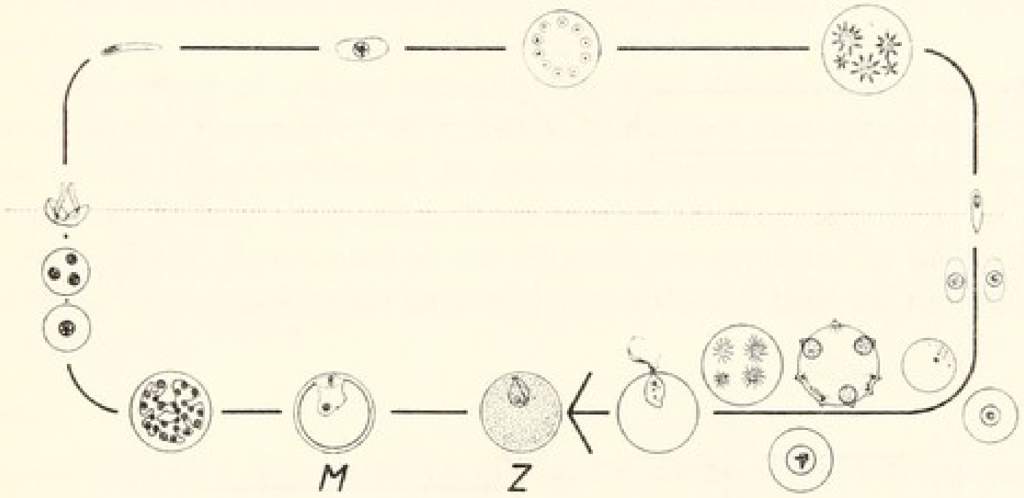

FIG. 2.2. The reproductive cycle in *Aggregata eberthi* (a sporozoon). The broken line marks the division between the part of the cycle which is carried on in the crab *Portunus* (above) and the part that is carried on in *Sepia* (below). M indicates the beginning of meiosis.

The spherical sporocyst has a thick membrane which, once it has been ingested by the crab, it opens and liberates three sporozoites. They make their way through the intestinal epithelium and reach the underlying connective layer. Each of them becomes a schizont, whose nucleus divides several times and thus a swarm of merozoites is developed from a single schizont. When *Sepia* eats an infected *Portunus*, the merozoites penetrate the epithelial intestinal cells and become gametocytes. Some of them give rise to a single big female macrogamete without undergoing any division, and some others originate numerous small male microgametes which are provided with two flagella and an undulating membrane. Fertilization gives rise to a big zygote.

Production of anisogamous gametes by apparently monoecious clones and the high specialization of the methods of agamic reproduction indicate the great possibilities of adaptation to parasitism of a haploid unicellular organism.

Haplo-diplonts

The highest degree of variability in life cycles is observed when meiosis takes place with spore formation in diploid asexual individuals and haploid sexual individuals are formed. A tendency to reduce the extent of the haplophase individuals has been observed in the evolution of different phyla.

1. Ectocarpus

The gametophytic and sporophytic individuals of the brown alga *Ectocarpus* resemble very much each other and are represented at maturity by branched filamentous tufts that only differ in respect of their reproductive organs (Fig. 2.3).

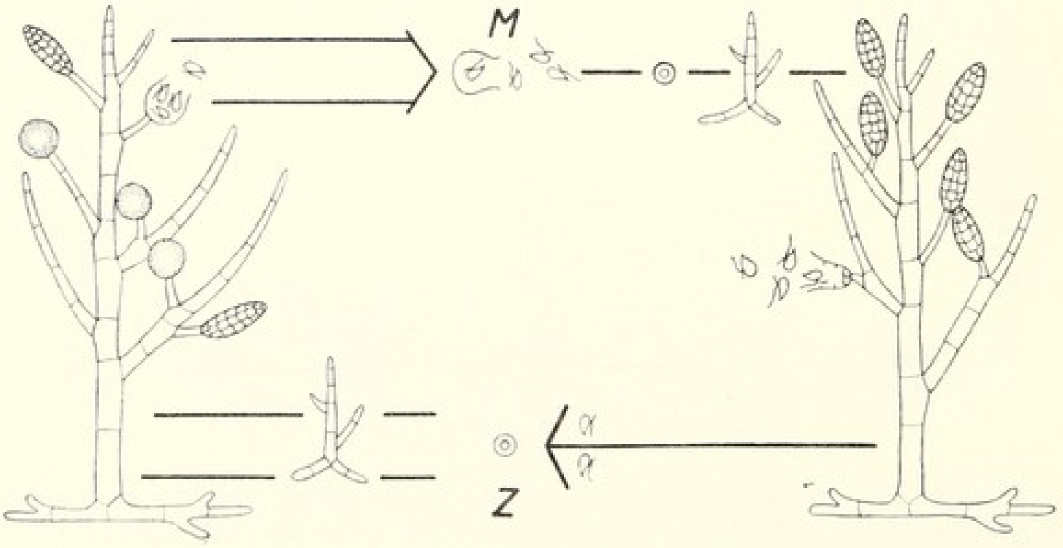

FIG. 2.3. The reproductive cycle in *Ectocarpus* (a phaeophyte). Z and M indicate the zygote and the outcome of the spore forming meiosis respectively. The haploid gametophyte generation and the diploid sporophyte generation are equally developed and they are enclosed within the single and the double lines respectively.

The non-motile zygote does not undergo reduction and develops a diploid plant (the sporophyte) which produces two types of sporangia on its lateral branches. Unilocular sporangia have an ovoidal form and begin with an enlargement of the terminal cell of a lateral branch. The single nucleus divides first meiotically and after that mitotic divisions form 64 or 128 nuclei which become the nuclei of the zoospores. They are provided with two lateral flagella of unequal length. Plurilocular sporangia are more elongated and repeated divisions give origin to hundreds of cubical locules where diploid zoospores are produced through a series of exclusively mitotic divisions. Such diploid zoospores from plurilocular sporangia produce new sporophytes.

The haploid zoospores, after they have reached a suitable solid object come to rest, loose their flagella and develop into the filamentous gametophyte through successive transverse divisions. The filamentous branch produces elongated gametangia where gametes are formed in the same way as in the elongated plurilocular sporangia of the sporophyte. The rupture of the gametangium liberates the gametes that are quite similar to haploid or diploid zoospores and unite in pairs to form the zygote. One of the gametes becomes immobile and the other remains motile before uniting. Differences between male and female sex are thus evident in this primitive brown alga. The zygote is then ready for development into a sporophyte. Although diploid and haploid plants are quite similar, the life cycles appear to be deeply influenced by racial and environmental influences which may eliminate the sporophyte generation from the cycle. Distinct male and female gametophytes are prevalent in the Mediterranean. Sporophytic reproduction appears to be prevalent in British and Swedish waters.

The phenomenon of relative sexuality has been described in *Ectocarpus silicolosus* where Hartmann (1925, 1937) established that gametes which function as male gametes toward the gametes of one plant may function as female gametes toward the gametes from another source (see p. 61).

The two gametes represented in the diagram are supposed to be derived from different male and female gametophytes although they are morphologically identical.

2. *Mosses*

The very schematic representation of Fig. 2.4 can be referred to a generalized type of moss, such as a species of *Bryum* or *Polytrichum*. The double lined section that in a diagram of *Ectocarpus* covered half of the life cycle to indicate the extent of the sporophytic generation is here reduced to a quarter of the whole cycle.

The sporophyte initially develops from the zygote inside the archegonium until the archegonium is broken by the rapidly elongating diploid embryo and is transformed in the calyptra that forms the cap of the sporophyte. This is differentiated into the foot that remains embedded in the gametophyte from which it absorbs water and nutritional material; into the long and slender stalk, named seta, and into the capsule which at maturity appears bent at the upper extremity of the seta. Each spore mother cell that lies in the central part of the young capsule produces by meiosis four spores as the capsule approaches maturity. The haploid spores, that are liberated from the sporophyte when the lid of the capsule drops off, germinate into gametophytes by breaking the outer layer of their walls.

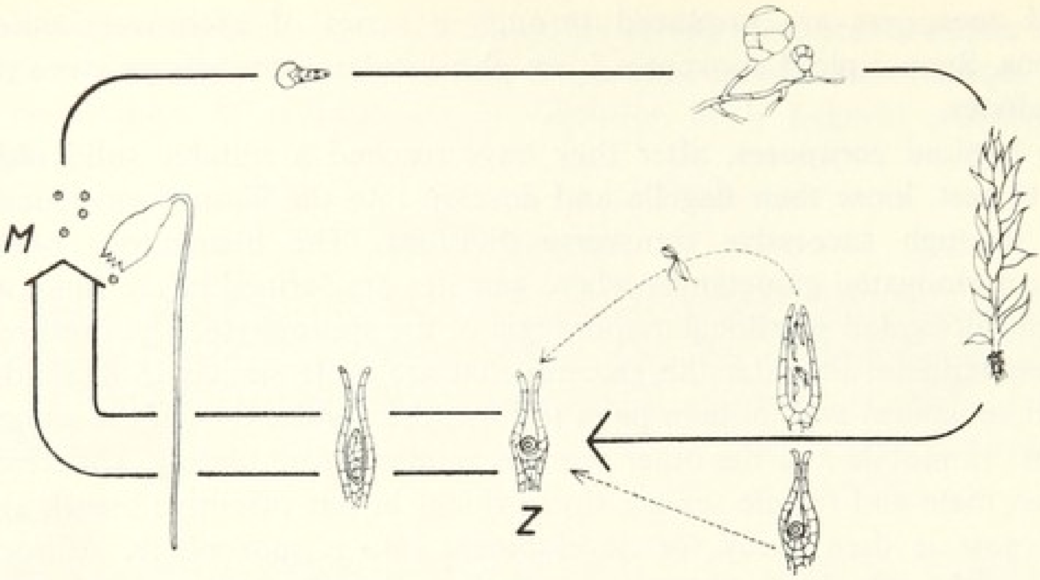

FIG. 2.4. The reproductive cycle in a moss. The sporophyte generation (within the double line) is not much developed.

A branching filament, the protonema, is formed and it gives rise to buds which in time grow into leafy shoots with rhizoids in their basal portion. Each bud becomes thus a moss plant which in time develops sex organs. The male organs, called antheridia, are rather slender and sack-like and enclose a number of male gametes (antherozoids) which have a slender somewhat coiled body bearing two long anterior flagella. The archegonia or female organs are flask-like and present an enlarged venter bearing a large egg which in the ripe archegonium communicates with the exterior through a canal of the neck.

The zygote is formed when the presence of water allows the antherozoids to swim and to penetrate the neck. In some species male and female organs are borne in different plants and in others they occur in the same group at the end of the stem or a branch. Dioecism and monoecism may occur within the same genus. Oogamy facilitates the production of the subparasitic sporophyte.

Mosses easily reproduce by vegetative multiplication and gametophyte and sporophyte may develop new plants by regeneration from little groups of cells.

3. Flowering Plants

In angiosperms the life cycle is dominated by the sporophyte and the male and female gametophytes have a short existence which is essentially associated with the process of fertilization.

The egg nucleus of the gametophyte is fertilized by the male nucleus within the ovarian walls of the sporophyte where the zygote begins its development into a new embryonic sporophyte. The development of the triploid endosperm, which surrounds and nourishes the young embryo, and the hardening of the ovule tissues into a seed coat contribute to the formation of seeds. When the seed has been shed free, either from ovaries or from fruits, it germinates into a seedling sporophyte which retains for a short time its embryonic leaves. The mature sporophyte is shown in the diagram as a generalized type of dicotyledon flowering plant (Fig. 2.5). Flowers (Fig. 2.6) are interpreted as highly specialized aggregates of both male and female sporangia (the anthers and the ovules), which are surrounded by the modified leaves known as sepals and petals.

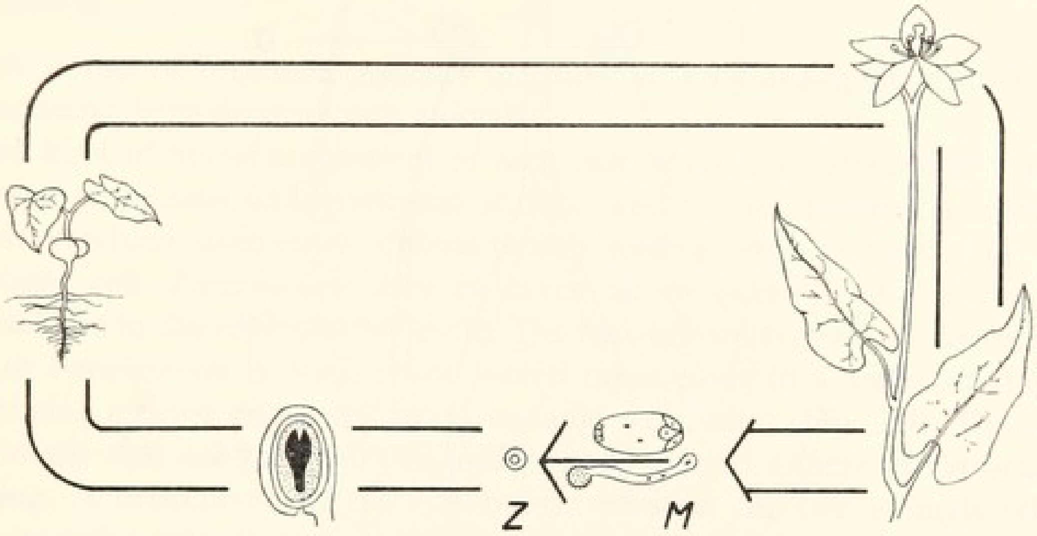

FIG. 2.5. The reproductive cycle of an angiosperm. The gametophyte generation, represented by the micro- and by the macrogametophyte where meiosis (M) occurs, is extremely reduced and develops within the parent sporophyte.

The female gametophyte (or macrogametophyte) grows inside the female organs of the flower that constitute the pistils. The pistil comprises the stigma, the style and the ovary which includes the already mentioned ovule. A single diploid megasporocyte is developed within an ovule and four haploid megaspores are formed through a meiotic process. Three of them degenerate and the remaining megaspore increases in size and develops into the female gametophyte which is produced by three successive mitotic divisions. The eight nuclei thus obtained have different destinies: three of them are enclosed in the antipodal cells at one end of the gametophyte, two form the so called fusion nucleus in the middle region and the three cells at the opposite end near the future point of entrance of the male nuclei are the synergids and the egg nucleus. The macrogametophyte is ready for fertilization at this stage.

The male organs of the flowers form the stamens with their stalk-like filaments and the terminal anther where numerous microsporocytes are formed inside the pollen sacs. Four microspores are formed by each microsporocyte through reductional divisions and each of them is functional and is able to give rise to a microgametophyte or pollen grain which is initially provided with two nuclei. When the pollen grain reaches the stigma it germinates a pollen tube which penetrates the female gametophyte while one of the two nuclei (the tube nucleus) remain undivided and the other (the generative nucleus) divides into two sperm nuclei. One of them, the male gamete nucleus, unites with the egg nucleus and forms the zygote, the other unites with the fusion nucleus thus giving rise to the triploid endosperm.

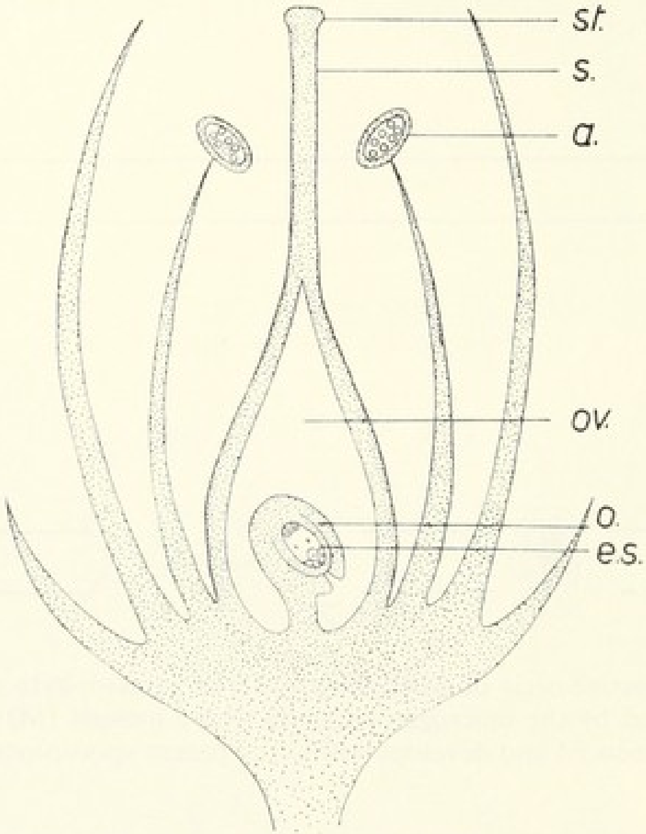

FIG. 2.6. Section of an hermaphroditic flower: e.s. embryo-sac; o. ovule; ov. ovary; s. style; st. stigma; a. anthers with pollen grains.

4. *Foraminifera*

In many species of Foraminifera a dimorphism of the shells has been observed: the first formed chamber is small in one form and larger in the other. It has been shown that the microspheric form develops from a zygote and it is diploid. The megalospheric form is haploid and it is generally produced by haploid amebulae from the asexual microspheric generation. The zygote is formed by the fusion of two flagellated isogametes.

Thus in Foraminifera alternance of sexual phase coincides with the alternation of nuclear phase.

Diplonts

Gametic reduction could be interpreted as the result of an evolutionary trend leading to extreme regression of the haplophase in the life cycle. In the genus of green algae *Cladophora* where haplont, diplo-haplont and diplont species coexist a similar process has probably taken place.

In Metazoa, where the gametes are the only known haploid cells of the normal cycle, no trace of a pre-existing independent haplophase generation has been detected. Thus it is impossible to state how the gametic reduction has become established in the numerous metazoan phyla.

Life cycles in Infusoria have been too little explored to allow any conclusion concerning the evolution of the diplophase.

1. Infusoria

This group of Protozoa presents the most striking examples of aberrant reproductive behaviour among animals.

Two kind of nuclei are present in each individual: a micronucleus that is regarded as an essentially germinal nucleus, and a macronucleus, a somatic structure which dominates differentiation and growth processes in the infusorial cell. *Paramecium* may be taken as an example of the various peculiarities in the reproductive cycle. The best-known type of sex reproduction in *Paramecium* is conjugation which takes place in a series of stages comprising meiosis and fertilization in rapid succession (Fig. 2.7). The two individuals that are going to conjugate approach and adhere at cytostome opening. A breakdown of the membranes between the two animals takes place and the cytoplasms intermingle. The macronucleus gradually breaks and dissolves while the micronuclei of each conjugant undergo meiotic processes and four haploid micronuclei are thus formed. Three of the four

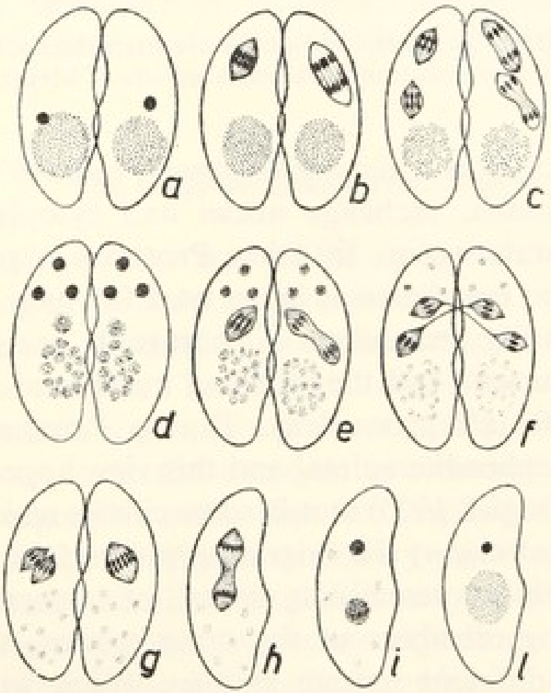

FIG. 2.7. The different phases of conjugation in a *Paramecium*.

nuclei dissolve in the cytoplasm and the single nucleus left divides by mitosis into two pronuclei, one of which migrates across to the other conjugant (Fig. 2.7f). Thus each *Paramecium* contains two pronuclei of different origin. Caryogamy takes place and the initial diploid number of chromosomes is restored in each individual. While the conjugants separate the diploid nucleus divides many times. One of the nuclei thus formed becomes the new micronucleus, and about forty of them fuse and form the macronucleus and the rest are dissolved in the cytoplasm. Thus the micronucleus has a diploid chromosome set and the macronucleus is highly polyploid.

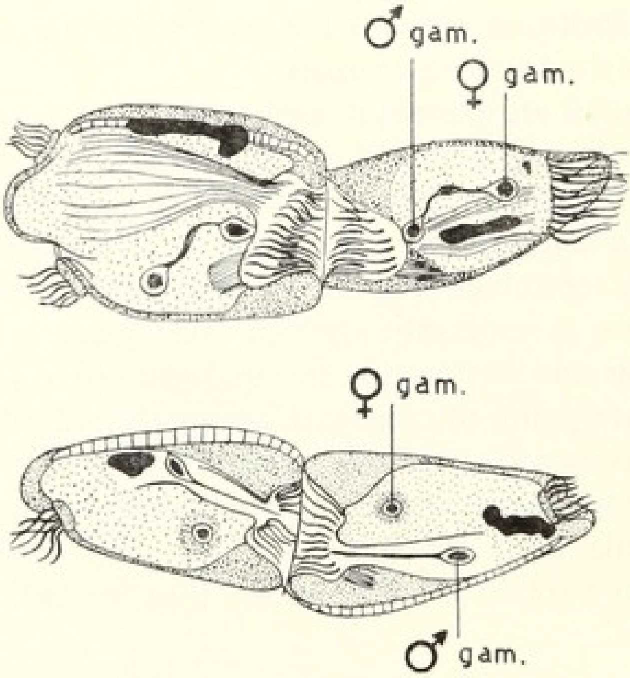

FIG. 2.8. In the conjugation of *Cycloposthium bipalmatum* the motile nucleus is provided with a plasmatic tail and it resembles the male gamete of Metazoa (Dogiel, 1925).

Conjugation differs from syngamy in other Protozoa for the reason that the conjugants approach, exchange nuclei and cytoplasmic material and after that they separate again. In other Protozoa a permanent fusion is obtained and the term copulation is being used for such normal fertilization processes. The migrating pronucleus of conjugants has generally been considered a male pronucleus and the standing nucleus of each conjugant has been considered a female pronucleus. Thus a *Paramecium* is commonly regarded as an hermaphrodite animal and this view appears to be supported by the observation (Dogiel, 1925) that in some ciliates which live as symbionts in ruminants (*Cycloporthium*) the migrating pronucleus is provided with a plasmatic appendix closely resembling the tail of a sperm (Fig. 2.8).

Conjugation among members of the same species is also regulated by their belonging to different mating systems which will be discussed in Chapter 3.

Autogamy also occurs in *Paramecium*. A single individual undergoes meiosis, caryogamy and dissolution of the macronucleus which is restored later. The only difference from conjugation is that caryogamy takes place between pronuclei from the same cell. Autogamy can therefore be compared to a case of internal self-fertilization. The advantage of this process seems to consist in the formation of a new macronucleus and in the establishment of new combinations of allelomorphic genes and such interpretation can also be applied to the already mentioned case of the Heliozoan *Actynophrys sol* which, according to Belar (1922), is also diploid.

The so-called process of endomixis has also been formerly reported for Infusoria by various authors. The macronucleus dissolves and it is restored by the micronucleus but neither meiosis nor fusion of pronuclei are said to take place in endomixis. Beale (1954) raises, however, serious doubts about the occurrence of such process in *Paramecium*.

The general advantages of conjugation appear to consist in the fact that the cytoplasm of each conjugant is enriched by exchange with foreign cytoplasm and by the breakdown of the macronucleus: new genetic combinations arise through the fusion of pronuclei from different sources.

2. Metazoa

The nuclear cycle of the Metazoa is very simple in spite of the extreme complexity of the diploid organisms. Gamete formation always leads to oogamy with spherical immobile egg cells, charged with great quantities of yolk material and with generally motile and always very small sperms which are devoid of reserve materials. The life cycles of the Metazoa are especially remarkable for the versatility of the sexual adaptations which cannot be described in a generalized cycle and will be therefore reviewed in special chapters.

The Haploid Dicaryotic Cycles

An example of this peculiar type of life cycle is presented by mushrooms (Fig. 2.9) that belong to the order of Hymenomycetes in the highly specialized class of Basidiomycetes. Two primary haploid mycelia develop undifferentiated cells, which fuse to form a binucleate cell or dicaryon (D) by a process of somatogamy or somatic copulation (Renner, 1916). The cells of the haploid mycelia that fuse to form the dicaryon are considered by some authors (Raper, 1954) as vegetative cells. The two haploid dicaryon nuclei divide simultaneously and thus develop a secondary mycelium which is composed by binucleate cells and is comparable therefore to a diploid mycelium. After a certain period of growth the secondary mycelium organizes to form a specialized portion called the fruiting body or basidiocarp.

The dicaryotic basidiocarp bears a unique type of sporangium called basidium, which in turn produces the basidiospores. Basidia of mushrooms are born on a palisade-like layer, the hymenium, are more or less cylindrical and have a rounded apex. The two nuclei of the dicaryon that gives rise to the basidium fuse and thus develop a diploid nucleus which migrates toward the apex of the basidium. Meiotic reduction takes place immediately and four daughter haploid nuclei are produced. They migrate into four slender projections at the distal end of the basidium. Each projection swells after penetration of the nucleus and four buds are formed that are sent off as basidiospores.

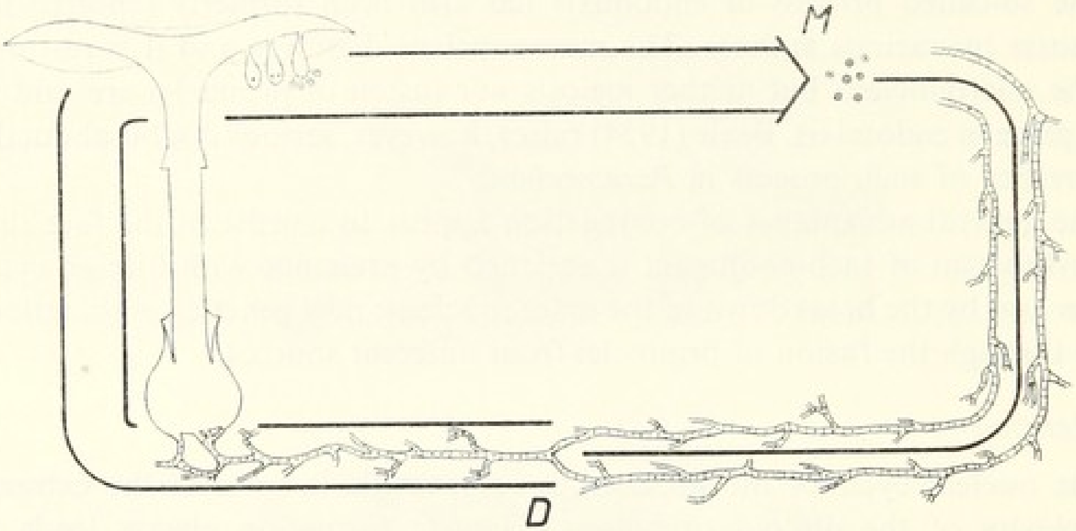

FIG. 2.9. The reproductive cycle of a basidiomycete. Somatogamy with dicaryon formation (D) originates dicaryotic mycelia, which correspond to the diploid generation of other organisms. Caryogamy and true zygote formation are immediately followed by meiosis and spore formation (M).

A haploid primary mycelium germinates from each basidiospore soon after discharge from the basidium when conditions are favourable.

It must be remarked that both the haploid and the dicaryotic phase are capable of indefinite growth in basidiomycetes.

The situation is different in the class of Ascomycetes and especially in its more specialized members.

In *Neurospora* the dicaryon phase is restricted to binucleate cells forming the so-called ascogenous hyphae.

In species of this group the two fusing elements, or gametangia, are developed by haploid mycelia and they may be sexually differentiated into more or less cylindrical antheridia and into oogonia or ascogonia which are usually prolonged into a trichogyne. Gametangial copulation takes place typically and the nuclei and cytoplasm of the antheridium migrate into the ascogonium where the nuclei of opposite sex approach without fusing. This type of fertilization, which is typical of the majority of ascomycetes, is effected in *Neurospora* by spermatia or microconidia which penetrate the trichogyne.

One or more ascogenous hyphae are developed from the fertilized ascogonia. They form at a given stage cross walls that separate binucleate cells. One of the nuclei originates from one of the male nuclei entering the ascogonium and the other originates from one of the ascogonial female nuclei. Each of the cells forms therefore a dicaryon.

Caryogamy takes place with the fusion of the two nuclei of the penultimate cell of each ascogenous hypha which has enlarged to many times its original size and has become club shaped. A meiotic division of the fusion nucleus follows immediately and after that the four nuclei resulting from meiosis divide again forming a row of eight nuclei in the ascus. Denser cytoplasm is next condensed around each nucleus and eight ascospores are developed by the formation of cell walls around each nucleus with the surrounding cytoplasm.

Mycelia derived from ascospores have haploid nuclei and they correspond therefore to the gametophytic generation. The haploid generation is therefore predominant in *Neurospora*, in *Penicillium*, *Aspergillus* and other higher ascomycetes: the separation in space and time of plasmogamy from caryogamy differentiates their cycle from that of pure haplonts.

The binucleate or dicaryotic phase assumes great importance in basidiomycetes and practically replaces the diploid or sporophytic phase in organisms that show zygotic reduction.

Life cycles in higher Fungi cannot however be sufficiently understood unless two peculiar processes are shortly mentioned: the Buller phenomenon (Buller, 1930) and parasexuality (Pontecorvo and Roper, 1952).

Buller first showed that somatogamy in basidiomycetes may take place between a haploid (or monocaryotic) and a dicaryotic mycelium and it may result in the dicaryotization of the haploid mycelium by one of the two nuclei in the cells of the dicaryotic mycelium. Legitimate combinations are formed when no incompatibility factors of the haploid mycelium appear to be duplicated with the penetration of the dicaryotizing nucleus, illegitimate combinations are developed when an incompatibility factor is duplicated by dicaryotization (Buller, 1941).

In case of legitimate combination a kind of fertilization of one fully developed mycelium by another takes place and a typical dicaryon is formed which becomes immediately capable of producing basidia and basidiospores. A type of mass fertilization takes place therefore in such instances. Illegitimate combinations on the contrary cannot give rise to typical dicaryons.

Parasexual processes (Pontecorvo, 1954) have been first identified by Pontecorvo and Roper (1952) in the ascomycete *Aspergillus nidulans* where genetic recombination takes place independently from the regular alternation of meiosis and caryogamy that takes place in the normal sexual cycle of the species. A parasexual cycle was later identified (Pontecorvo, 1953; Pontecorvo, Raper and Forbes, 1953) in the otherwise completely asexual species

Aspergillus niger and in some other sexual and asexual species of ascomycetes. Haploid nuclei of unlike genotype may come together to form a heterocaryotic mycelium and a few of them (in a proportion of 10^{-6}) fuse to give a diploid heterozygous nucleus. A haplo-diploid heterocaryotic mycelium is thus formed with the diploid nuclei multiplying side by side with the haploid nuclei. During the multiplication of the diploid nuclei (which may be sorted out) mitotic crossing-over takes place in a very low percentage and finally a small proportion of diploid nuclei undergo haploidization. Haploid nuclei, some of which are recombinant, are thus obtained and they may develop haploid strains where recombination can be recognized by means of suitable genetic markers. Thus the parasexual cycle contributes to gene recombination in species where the sexual cycle is present and is the only source of genetic recombination in species totally lacking typical sexual processes.

Nuclear Phases and Sex

A survey of sex conditions in haplo-diploid organisms lead to the conclusion that also when the haploid phase individuals are unisexual the diploid phase individuals show a marked hermaphrodite tendency, which can be shown through appropriate experimental devices.

A brilliant series of experiments carried on by the Marchals in some species of dioecious mosses of the genera *Bryum* and *Mnium* more than 50 yr ago (1906–1907) gave early support to this view (Fig. 2.10). Each spore proved to be either male or female producing in isolation experiments and external influences such as changes in nutrition, in light, heat or moisture, failed to produce the least effect on the sex phenotype. The Marchals obtained new gametophytes from small fragments of the stem or of the leaves and they were of the same sex as the original plants (Fig. 2.9, first row).

The Marchals were also able to obtain new moss plants, similar in appearance to the gametophyte, but diploid in nuclear constitution, by regeneration from small pieces of the sporophyte taken either from the sporangium or from the stalk (Fig. 2.10, last row). Many of such diploid plants were sexually hermaphrodite although some of them resulted in a prevalently male or female condition.

Such results demonstrated that: (1) the male and female producing factors are associated in the diploid cells of the sporophyte, (2) they are segregated during the meiotic divisions of spore formation and (3) they are brought together again in fertilization.

The Marchals' work provided thus one of the earliest experimental results in support of the genetical interpretation of sex determination and introduced at the same time a useful method in the comparative study of sex in the haplo- and in the diplophase.

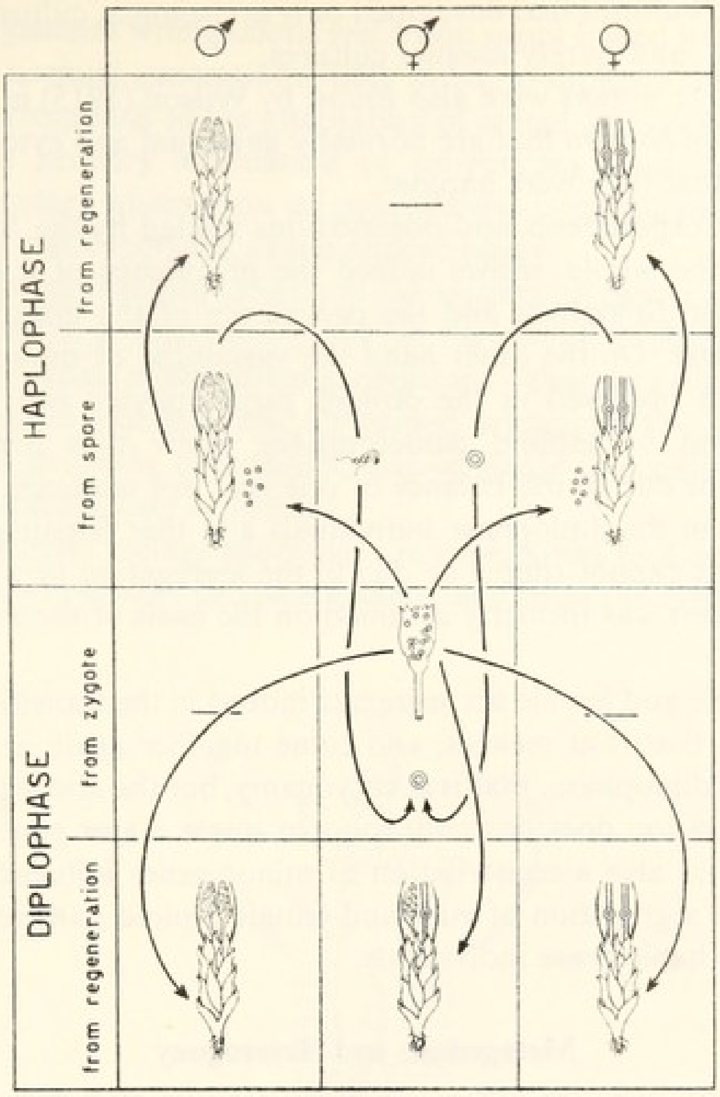

FIG. 2.10. Nuclear phases and sex in dioecious mosses. Haploid gametophytes obtained through regeneration from other gametophytes are either males or females, diploid gametophytes obtained through regeneration from the sporophyte are male, female and hermaphroditic (after Marchal, 1909–1910).

Schweitzer (1923) confirmed in *Splanchnum sphaericum* the Marchals' results by producing hermaphrodite and pure male and female diploid gametophytes from phragments of the sporophyte.

F. V. Wettstein obtained (1924a) diploid gametophytes of *Bryum cespiticium* which formed at first purely male, then hermaphrodite and lastly a few purely female gametangia. Ratios between male, hermaphrodite and female gametangia were 7·3 : 2·0 : 1·5 respectively and proportions of antheridia to archegonia resulted 5 : 1 in hermaphrodites.

Wettstein was also able to cross diploid with haploid gametophytes and obtained triploid gametophytes that presented protogynous hermaphroditism.

More recent research by Hoffmann (1956) has shown that diploid gametophytes of *Bryum capillare* are always hermaphrodite and on the other hand regeneration experiments from sporogonia of *Barbula unguiculata* (Hoffmann,

1957) produced cultures that developed only archegonia, cultures that showed only antheridia, and purely asexual cultures.

Hermaphrodite organs were also found by Wilson (1915) in a few gametophytes of *Mnium hornum* that are normally unisexual and cytological control demonstrated that they were haploid.

The series of experiments and observations started by the Marchals, when considered in the whole, shows indeed the prevalence of a hermaphrodite condition in the diplophase and the prevalence of the unisexual condition in the haplophase. On the other hand the variability of the sex phenotypes, which has been observed in the diploid gametophytes and the reports of hermaphroditism in haploid gametophytes, show that hermaphroditism cannot simply be due to the balance of one gene for maleness with one gene for femaleness in the diplophase individuals and that female or male sex in the gametophyte cannot simply be due to the segregation of a single male or female factor as it was formerly assumed on the basis of the early mendelian interpretations.

Genes for male and female sex segregate indeed in the transition from diplo- to haplophase, that is at meiosis, and come together again in the transition from haplo- to diplophase, that is at caryogamy, but the above results indicate also that segregation does not only concern single major genes or blocks of genes for sex but also a constellation of minor genes influencing sex which account for the segregation of male and female diploid gametophytes and of hermaphrodite haplophase individuals.

Metagenesis and Heterogony

The alternance of nuclear phases in organisms undergoing zygotic reduc-

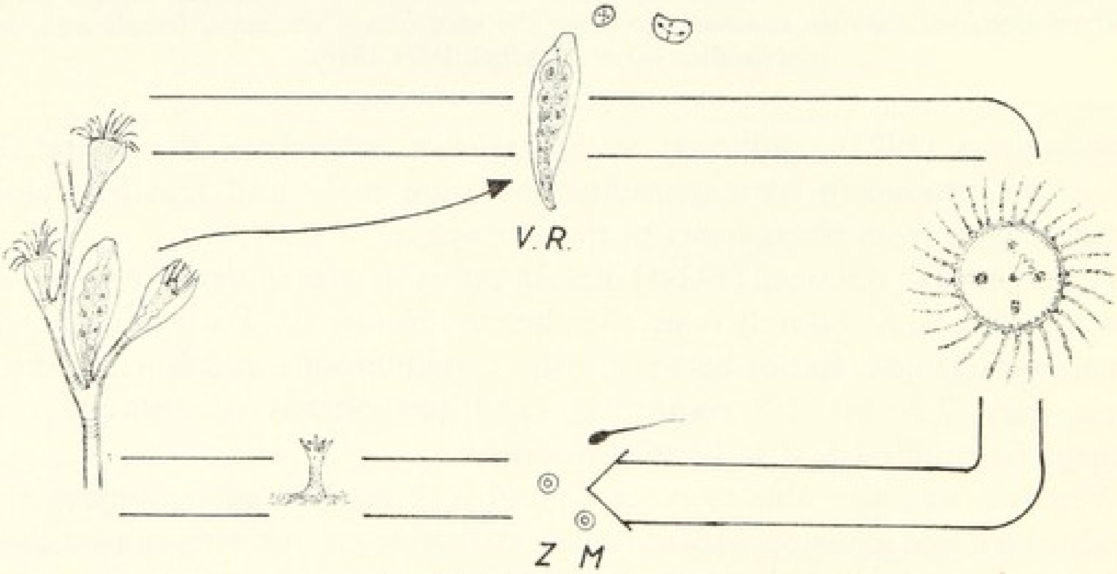

FIG. 2.11. The reproductive cycle of *Obelia*, a coelenterate. Gametic reduction takes place (M) and the sexual generation represented by the medusa arises by vegetative reproduction (V.R.) from the sessile asexual generation.

tion and in organisms with gametic reduction is not linked with an alternance of sexual and asexual generations; only in haplo-diplonts the change of nuclear phase corresponds to the alternation of sexual and asexual generations. Therefore this primary alternation of generations must be distinguished from the secondary alternation of generations or metagenesis which is accomplished by some metazoa in the diploid phase.

Metagenesis was discovered early in the nineteenth century by the German poet and naturalist v. Chamisso (1819) in the pelagic tunicates of the genus *Salpa*: the individuals of the sexual generations—that are called blastozoids—are hermaphrodite and are typically united in chains. The zygotes develop into solitary individuals, or oozoids, that originate the chain forms through the vegetative production of stolons.

Other well known metagamic cycles are exemplified by Coelenterates (Fig. 2.11): the pelagic medusae produce gametes and from the zygotes develop the

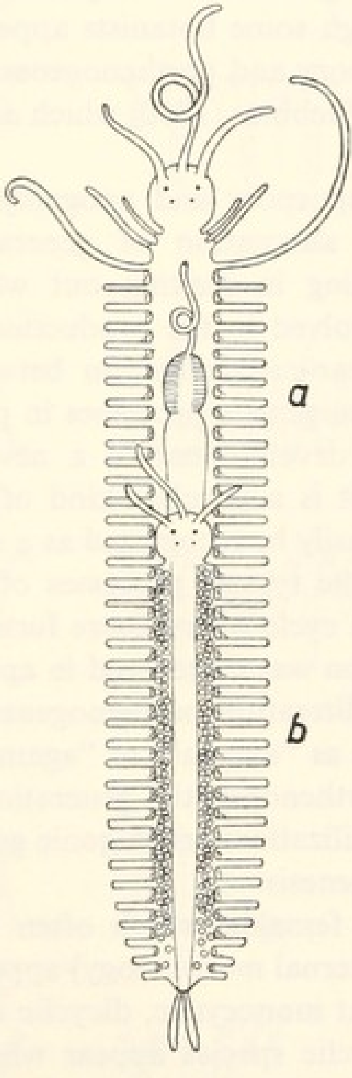

FIG. 2.12. A polychaete of the syllid family which is reproducing asexually. The epitokous part (b) carries eggs and will reproduce sexually.

sessile polyps which remain isolated or give rise to colonies of polyps which remain connected by a system of tubes that form the coenosarc.

Metagenesis takes place also among polychaetes. Some species reproduce sexually with their epitochous pelagic generation which is produced through stolonization by a benthic agamic generation (Fig. 2.12).

Metagenesis usually designates the regular alternation of an asexually and a sexually reproducing generation but irregular cycles often take place and some of the environmental factors determining sexual reproduction have already been enumerated.

In regular metagamic cycles the agamically reproducing individual produces the sexual individuals according to genetically fixed rules. This has been demonstrated by a work of Cognetti (1956) which showed that in the polychaete worm *Autolytus benazzii* male or female stolons constantly sprout in all individuals either on the 22nd, the 26th, the 30th, or on the 34th setigerous segments (Fig. 2.13).

The resemblances existing between metagenesis and heterogony are more apparent than real although some botanists appear inclined to group vegetative reproduction, apospory and parthenogenesis within the vast category of apomictic phenomena (Stebbins, 1950) which are said to be the equivalent of asexual reproduction.

The various kinds of apospory and apogamy, the complication of the sporophyte gametophyte alternation of generation combined with the technical difficulties existing in making out whether somatic tissues or germinal elements are involved in the production of new individuals have sometime prevented a clear-cut distinction between asexual reproductive process and sexual parthenogenetic processes in plants.

Parthenogenesis is the development of a new individual from an egg without fertilization and it is a peculiar kind of sexual reproduction. The parthenogenetic egg can easily be recognized as a sexual cell among Metazoa as it undergoes most of the typical processes of oogenesis and cannot be confused with a spore in a cycle where spore formation is absent.

This kind of reproduction was discovered in aphids by Bonnet (1762) and in the current zoological literature parthenogenetically reproducing individuals are often referred to as "asexual" or "agamic" females.

The alternation of parthenogenetic generations with generations that reproduce by means of fertilization (amphigonic generations) is called heterogony or cyclical parthenogenesis.

Males and amphigonic females (which often differ from the parthenogenetical females in the external morphology) appear one, two or more times during annual cycle so that monocyclic, dicyclic and polycyclic populations have been described. Acyclic species appear when males and amphigonic reproduction have been eliminated from the life cycle: evolution leads thus to purely parthenogenetic reproduction.

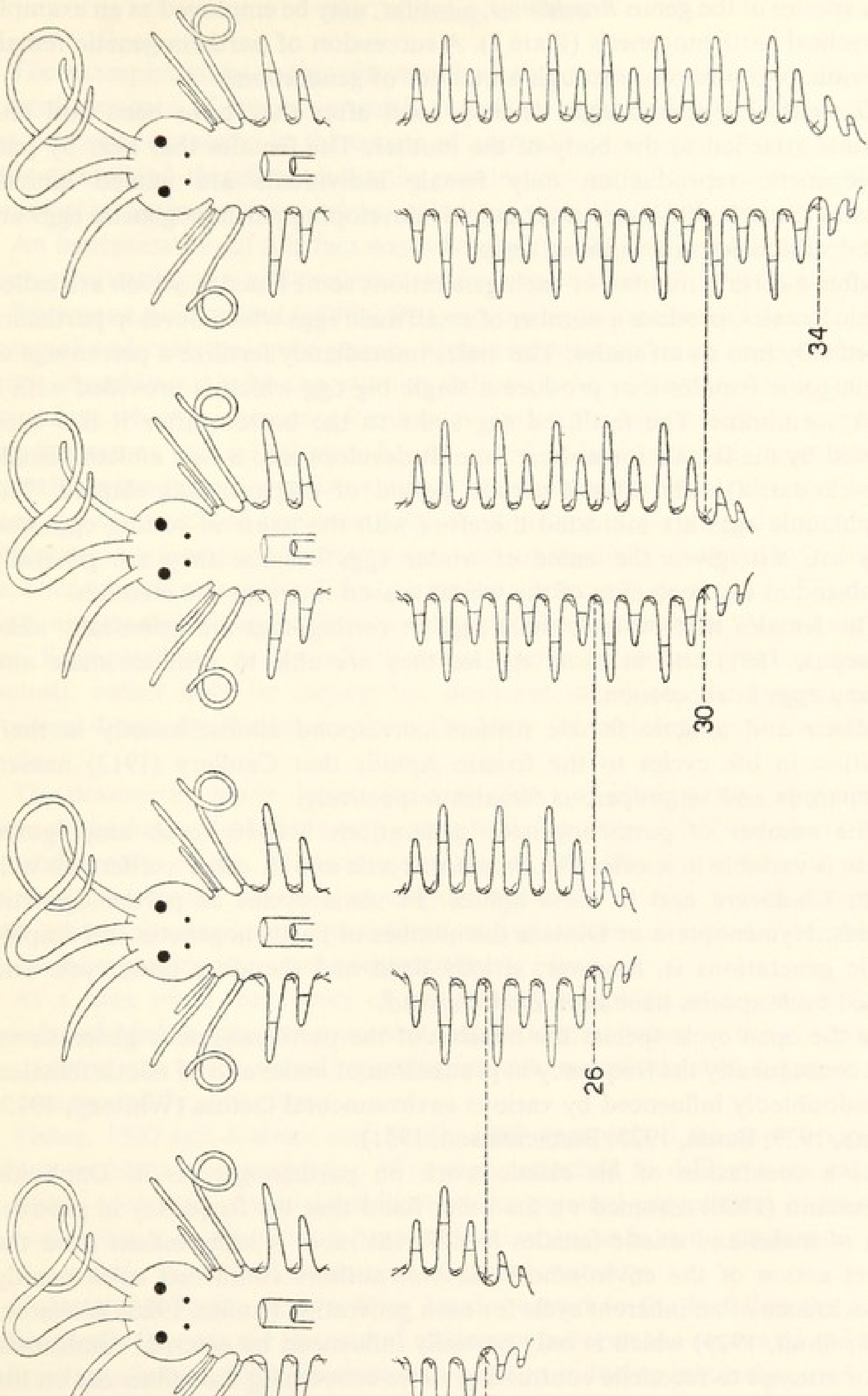

FIG. 2.13. Stolonization of *Antolytus benazzii*: it takes place at fixed number of segments (Cognetti, 1956).

A species of the genus *Brachionus*, a rotifer, may be employed as an example of cyclical parthenogenesis (Plate I). A succession of parthenogenetic female individuals is observed through a number of generations.

The parthenogenetic eggs develop soon after they have been laid and become attached to the body of the mother. The females that bear by parthenogenetic reproduction only female individuals are named amictic females (Storch, 1924) and their quickly developing parthenogenetic eggs are called subitaneous (or summer) eggs.

After a certain number of such generations some females, which are called mictic females, produce a number of small male eggs which develop parthenogenetically into dwarf males. The males immediately fertilize a percentage of amphigonic females that produce a single big egg which is provided with a thick membrane. The fertilized egg sinks to the bottom after it has been carried by the female for a short time. It develops into a new amictic female (the fundatrix) only after a certain period of diapause has elapsed. The amphigonic eggs are indicated therefore with the name of resting eggs and they are also given the name of winter eggs because they are generally produced at the beginning of the winter season in temperate climates.

The females that produce male eggs or resting eggs are genetically alike (Maupas, 1891) and in some species they are able to produce male and resting eggs in succession.

Mictic and amictic female rotifers correspond almost exactly in their position in life cycles to the female Aphids that Caullery (1913) named sexuparous and virginiparous females respectively.

The number of parthenogenetic generations between each amphigonic phase is variable in species like *Brachionus pala* and in other rotifers as well as in Cladocera and in most aphids. In some cycles of parthenogenetic aphids, Hymenoptera or Diptera the number of parthenogenetic and amphigonic generations is, however, strictly fixed and therefore open cycle and closed cycle species have been distinguished.

In the open cycle species the number of the parthenogenetic generations, and consequently the frequency in production of males and of mictic females, is undoubtedly influenced by various environmental factors (Whitney, 1912; Luntz, 1929; Banta, 1925; Bonnemaison, 1951).

As a conclusion of his classic work on parthenogenesis in Daphnids Weismann (1880) assumed on the other hand that the frequency in production of males and mictic females in different races is independent from the direct action of the environment. Several authors confirmed subsequently the existence of an inherent cycle for each generation (Luntz, 1926; Wolterek, 1928; Shull, 1929) which is only partially influenced by external conditions.

An attempt to reconcile contrasting views concerning the influences on the heterogonic cycle (Bacci, 1957) will be discussed after the cytological and sexual situation of parthenogenetic cycles has been examined in more detail.

The Meaning of Sex

The examples given in this chapter show that sexual reproduction implies a rigid sequence of processes which are rather complicated as compared to the processes occurring in the course of asexual reproduction. Many authors have speculated therefore about the reason why sexuality is so much more widespread among plants and animals than asexual reproduction.

An interpretation of this fact was advanced by Weismann about 70 yr ago soon after caryogamy and meiosis were discovered by the German cytologists.

Weismann pointed out (1886a) that caryogamy brings about the mixing of paternal and maternal germplasms, a phenomenon which he named amphimixis. Amphimixis helps therefore to diffuse the hereditary variability of the organisms which is necessary for their evolution through selective processes. For this reason natural selection has favoured, in Weismann's interpretation, sexually reproducing organisms and purely asexual reproduction has been confined to lower and comparatively small groups of plants and animals.

The explanation given by Weismann was substantially accepted by modern genetics when it was realized that germplasm is actually composed of discrete units or genes which are subject to mutation. The fact that the "Vermischung der Keimplasmen" is actually brought about by recombination during meiosis, rather than by caryogamy, does not invalidate the general conclusions drawn by Weismann from the early information upon the sexual cycle.

The discoveries made during the early 30 yr of investigation in genetics were at the basis of Muller's very important discussion (1932) on the fundamental aspects of sex. Muller's view that sex provides the means for using in the best way the possibilities of gene mutations is now widely accepted and it has helped in laying the theoretical foundations of such discoveries as bacterial recombination and parasexuality in Fungi.

At a time when the theory of genetic polymorphism was not yet fully developed Muller attached comparatively little importance to the evolutionary advantages which sexual reproduction can provide through heterosis, as first suggested by Altemburg.

Fisher, 1930 and Kalmus and Smith, 1960 approached the comparatively minor problem of the selective advantages attached to different sex ratios.

Fisher took into special consideration the parental expenditure in rearing the offspring, a result which is best attained when, as in many mammals, the 1 : 1 sex ratio is reached at the time of sexual maturity.

Kalmus and Smith, on the other hand, pointed to the fact that the 1 : 1 ratio greatly favours the chances of encounter between the sexes and reduces inbreeding. Sexual differentiation also helps in reducing inbreeding and has therefore been favoured by natural selection.

It can be noticed at this point that incompatibility (see Chapter 3)—which is the opposite of sex differentiation—reduces inbreeding and favours heterosis. The study of processes involved with sexual reproduction actually shows many instances of different systems leading to quite similar results.

It is difficult therefore to evaluate properly the actual role of certain biological processes in evolution and a cautious attitude must be recommended when dealing with general evolutionary problems. Critical viewpoints must also be given the utmost attention.

Some authors contend (Canella, 1959) that the amphimixis theory and its recent enunciations totally lack experimental support and that some very successful groups of organisms reproduce without showing any trace of sexual processes. It must be pointed out, on the other hand, that sexual or parasexual phenomena are constantly being discovered in new organisms and that—in Weismann–Muller's theory—sexual reproduction is not said to be a necessity for the mutating plants and animals but only to confer great advantage to sexual over asexual organisms.

It is also quite true that a number of panglossian speculations have been unduly advanced by a few followers of the amphimixis theory but it cannot be forgotten that its pragmatic value has received confirmation in the recent discoveries of sex processes in bacteria and viruses.

Hartmann (1909, 1956) followed personal views and regarded sex as a basic property of the living matter which appears to be necessarily linked with fertilization: every organism, in Hartmann's interpretation, is endowed with a bipolar sexuality and with the potencies for male and female sex. This line of thought, which practically ignored the solution given by Weismann, stimulated a fruitful search for sex differentiation in organisms where purely morphological investigation had been unable to find any trace of sex polarity.

The observation that copulation in protists, conjugation in Infusoria and gametogenesis in the few metazoa which usually reproduce through vegetative processes, can be induced by a number of external agencies, gave rise to rejuvenation, hunger and metabolic theories of sex. Such theories, although they have been supplanted by the genetic theory of sex, should be recalled when the problem of how sex has arisen, will be ripe for experimental investigation.

CHAPTER 3

SEX IN LOWER ORGANISMS

Recombination and Differentiation in Protosexual, Eusexual and Parasexual Cycles

MANY observations and experiments discussed in this chapter will appear rather disconcerting to the biologist who has concentrated his attention upon sex processes occurring in the so-called higher organisms. Striking differences are observed on the other hand also between sexual processes occurring in different phyla that are included under the common denomination of lower organisms.

The function of bringing together the genes from different sources in one cell lineage is carried on in a basically uniform way in the sexual cycles that have been described in the previous chapter. Meiosis and caryogamy follow the same fundamental schemes in phytoflagellates as well as in angiosperms and in vertebrates, zygotes inherit the complete chromosome sets both from male and from female gametes. The exchange of genetic material always takes place in the way described for meiosis.

The processes that replace the sex cycle of all other organisms in bacteria and viruses show, on the other hand, some peculiarities that point to a strong contrast with the sex processes described so far and recombination seems to be—at the present stage of research—the only basic feature that is common to sex phenomena occurring in bacteria and in all other organisms.

The so-called sex differentiation of bacteria, although it resembles sexual differentiation in higher organisms (Hayes, 1960a) is, in the author's opinion, by no means homologous to differentiation into male and female or even into + and − sex, which is shown by other organisms, including some unicellular algae or some fungi, that are mentioned in this chapter. Wollmann and Jacob's researches (1958) gave also strong indications that factors determining sex processes in bacteria are different from factors responsible for sex determination in other organisms. It must be concluded therefore that amphimixis, and thus sex, occurs in bacteria as well as in all other types of organisms but that sexual differentiation and its genetic determiners are by no means the same.

It is suggested for the above reasons to distinguish protosexual processes, occurring in bacteria and viruses, from eusexual processes and to leave the name of parasexual processes (Pontecorvo, 1954) to somatic crossing-over and haploidization occurring in some fungi. Sex is brought about in bacteria by mechanisms which appear to be primitive when compared to meiosis and caryogamy in eusexual organisms. A distinction between protosexual and parasexual processes is regarded on the other hand as useful when considering the great differences existing between the bacterial and the fungal chromosome and also when considering that mitotic crossing-over and haploidization seem to represent secondary adaptations in organisms like ascomycetes that have evolved from phyla where an eusexual type of cycle had been originally well established.

Difficulties in understanding sex cycles in lower eusexual organisms are due to a different set of reasons.

To begin with, our present knowledge of life processes in protozoa, algae and fungi is very fragmentary indeed and it may lead to serious misinterpretations.

The main difficulties lie, however, in the fact that some mechanisms, which appear as closely integrated in the reproductive systems of higher organisms, take different and sometimes divergent paths in the life cycle of some lower organisms. They appear thus like disintegrated elements of the apparently simple but essentially complicated sexual systems of the higher organisms. An example of this is provided by the temporal separation between plasmogamy and caryogamy occurring in the haplodicaryotic cycles of some fungi.

A difficulty of opposite type is met when dealing with sexual and incompatibility systems: it is easy to distinguish such systems in higher plants but big difficulties arise in understanding their relationships among fungi or among Infusoria. Some ciliates are unable, for instance, to conjugate with individuals belonging to the same mating type but they are able on the other hand to undergo autogamy: it is as if a *Nicotiana* plant, which cannot be fertilized by the pollen grains of another plant carrying the same sterility alleles, were capable of self fertilization.

Observations on the so-called relative sexuality lead to question even the existence of the clear-cut distinction which is generally observed between the two sex categories. The problems which are still open on sex in lower organisms are indeed far more numerous than those that have found satisfactory solutions.

Only a few examples have been chosen in order to illustrate some aspects of sexuality in lower organisms. They concern chiefly the sex factor in bacteria, the so-called relative sexuality in some algae and fungi, the incompatibility systems in Infusoria and a few other minor items.

Protosexual Processes in Bacteria

1. Bacterial Conjugation and the Nature of the F Factor

Bacteria were generally considered 20 yr ago as asexually reproducing organisms but genetic recombination, that follows the so-called conjugation, was demonstrated in 1946 by Lederberg and Tatum in *Eschericia coli.* Hayes discovered in 1952 marked differences (which recall sexual differentiation) between the members of the pairs that undergo conjugation.

Morphological research has helped very little indeed in understanding exchange of genetic material between different strains of bacteria. Although discrete organized chromatin bodies have been detected in bacteria no true mitotic processes have been demonstrated and genetic analysis has given no evidence that bacteria and viruses possess a classical type of meiosis.

Convincing pictures of actual matings between different strains of *Eschericia coli* were taken by Anderson (1958) and they showed formation of bridges between bacterial cells of different origin that could be identified by means of a filament or other morphological markers (Fig. 3.1).

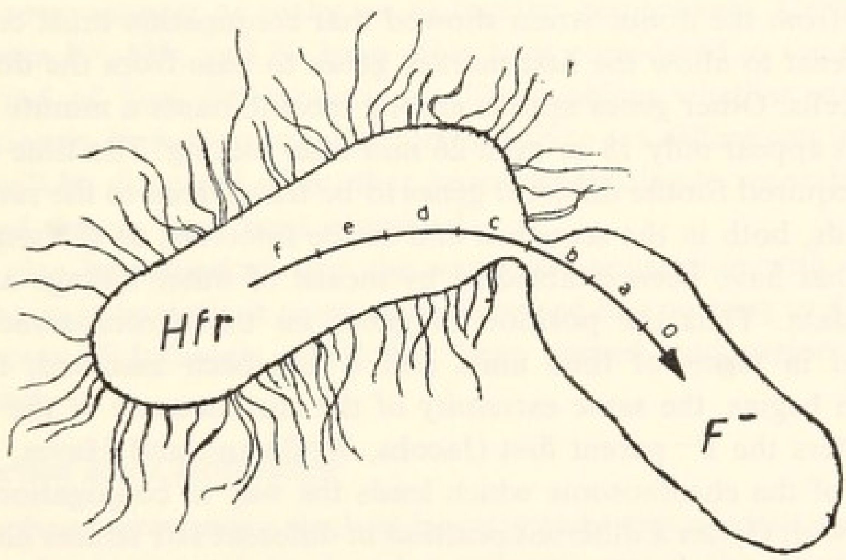

FIG. 3.1. A diagram of bacterial conjugation: the chromosome of the donor Hfr bacterium is introduced into the recipient F$-$ bacterium from a fixed point of origin (O).

The work of Lederberg and his collaborators was carried on along the same lines as those which were devised for the chemical genetics of *Neurospora* and mutants for certain nutritional deficiencies were used as genetic markers. Such genetic properties as resistance to certain bacteriophages or to antibiotics are now being more and more widely employed in bacterial genetics.

It was soon realized that not every strain will conjugate with every other strain but that conjugation can take place between bacteria belonging to two different mating types which were indicated as F$^+$ and F$^-$ respectively. Recombinants were not obtained in attempts to cross F$^-$ strains with each other but they were obtained after matings with the F$^+$ strains. Although the frequency of the recombinant types is very low in normal strains (of the order of 10^{-6}), frequencies of recombination as high as 0·5 or 0·01 were obtained with the discovery of Hfr (for high frequency) strains in some F$^+$ stocks.

A number of researches has shown that only the F^- strain gives recombinant progeny and that the F^+ or Hfr parent strains continue to produce their original genotype exclusively. The transfer of the genetic material is therefore unidirectional from F^+ or Hfr to F^- (Hayes, 1952) with the cells of the F^+ or Hfr strains acting as donor and the cells of the F strain functioning as recipient cells. It was also established that only one part of the chromosome of the donor cell is generally transferred to the recipient cell (Hayes, 1953) and therefore conjugation results in the formation of a merozygote and not in the formation of a holozygote as in eusexual organisms.

A further outstanding contribution to the analysis of bacterial recombination was made when Jacobs and Wollmann (1958) introduced the technique of interrupting the conjugation of mating cells at different time intervals after mixing. The analysis of the recombinants obtained for inheritance of different genes from the donor strain showed that conjugation must continue for 8 min at least to allow the first marker genes to pass from the donor to the recipient cells. Other genes appear among recombinants a minute or so later and others appear only 18 or even 26 min after mixing. The time in minutes which is required for the different genes to be transferred to the receptor cells corresponds, both in the sequence and in the intervals, to the sequence and distance that have been established by means of other linkage and recombination data. Thus the position of genes on the chromosome has been established in terms of time units and it has been assumed, that, when copulation begins, the same extremity of the chromosome of the F^+ parent always enters the F^- parent first (Jacobs, Wollmann and Hayes, 1956). The extremity of the chromosome which leads the way at conjugation, which is indicated with O, has a different position in different Hfr strains although the genes are arranged in the same sequence and at about the same distances. This situation has been interpreted by assuming that O represents the end of rupture of a circular chromosome where genes are arranged in the same way.

It is very significant from the point of view of mating type determination that in the case of $F^+ \times F^-$ crosses all the F^- cells inherit the donor character and are rapidly converted to F^+ type (Cavalli and Lederberg, 1953). The $F^+ \times F^-$ crosses are fertile.

Crosses between Hfr donor cells and F cells produce on the other hand very few cells with the donor character among the recombinants. The spontaneous change from F^+ to Hfr state, which seems to be the main factor of the fertility of the F^+ strains reduces the ability to transmit the donor state to F^- cells while greatly increasing the frequency of recombination.

The nature of the factor inducing the donor state, the so-called F or fertility factor, has been especially investigated by Jacobs and Wollmann who introduced the notion of episome (1958). The episome is regarded as a genetic element which is a non-essential constituent of the bacterial cell where it may exist either as an integrated unit attached to the chromosome with which it reproduces or as an autonomes unit reproducing independently of the bacterial chromosome. According to this interpretation the F factor is anchored in the Hfr strains to a regular locus of the bacterial chromosome and behaves as a chromosomal constituent of the host cell: the F locus is at the end opposite the O point. In the F^+ strains, on the contrary, the fertility factor behaves as though it were in an autonomous state and multiplies at least twice as fast as the typical cell. This explains why the exconjugants and progeny of $F^+ \times F^+$ crosses become F^+, that is are infected with the F factor. It is remarkable that the F factor, which behaves very much like a temperate phage, represents the first known example of extra-chromosomal genetic material which plays a decisive role in a normal reproductive process.

Donor (F^+ and Hfr) cells and recipient (F^-) cells are now often called "male" and "female" cells because, as first pointed out by Hayes (1952) they behave in some respect as males or as females respectively. Certain differences between F^+, Hfr, and F^- have often been considered as the expression of some kind of "sex differentiation". The problem whether mating type differentiation in *Eschericia* actually correspond to sex differences in eusexual organism will be discussed after other processes leading to recombination in bacteria and viruses have been examined.

It must also be remarked that the name of conjugation with which the mating process in bacteria is commonly described has nothing to do with the sexual process of Infusoria which has been named conjugation these last 50 yr at least.

2. *Transduction and Lysogenation*

Bacteriophages are among the best investigated types of virus and they are composed of a hexagonal head and a tail with which the phage attaches itself to the bacterial wall. The protein coat of the phage encloses DNA in the shape of a long thread and remains outside the bacterial body when the nucleic acid content has been injected into the bacterium.

When a virulent phage infects a bacterium it reproduces within them until the bacteria are destroyed by lysis.

The so-called temperate phages, however, enter the bacteria and establish a sort of stable association with the host by attaching themselves to a specific site of the bacterial chromosome. They constitute in this way an integrated unit of the chromosome and are inherited as such by the progeny of the bacterium. Phages thus transformed are called prophages and, as their nucleic acid threads are quite short, they behave approximately as a new gene on the chromosome of the bacterium and are generally indicated by a Greek letter on the genetic map of the bacterium. Only a small proportion of the so-called temperate phages enter the lytic cycle and reproduce as autonomous units.

The ability to produce and maintain the temperate phages in non pathogenic condition is inherited by the host bacteria which are called lysogenic bacteria.

Zinder and Lederberg demonstrated in 1952 that when a mutant strain was infected by phage propagated on the wild type, the cells which survived the phage infection showed hereditary qualities of the wild type strains. Recombination between different strains of bacteria is effected therefore by means of genetic vectors. The process operated by phages that become able to transfer parts of the chromosome from a dissolved bacterial cell and to introduce them into other bacterial cells is called transduction (Fig. 3.2). There is no available data to explain how the genetic material from the donor strains is integrated within the chromosome of the recipient strains and ultimately segregated to the progeny, as direct tetrad analysis is impossible in bacterial genetics. For this reason diagrams concerning such processes in transduction are very approximate.

There are in transduction, as in conjugation, a donor cell and a recipient cell. The transduced section of the bacterial chromosome is always very short so that the recipient cell inherit only a genetic locus and occasionally two loci. This allows one to build genetic maps and to carry on a detailed analysis of the structure of the bacterial chromosome.

Transduction can be regarded as a sexual process as it brings about a mixing of genetic material from different strains but comparisons with sexual differentiation in eusexual organisms is even more remote than in conjugation. The comparison of temperate phages, which are episomic elements, with the F factor appears on the contrary quite convincing.

The process of transformation in which a bacterial strain can be genotypically changed by the introduction of DNA from other types of bacteria and recombination in bacteriophages are now being actively investigated. Such investigations have, however, yielded as yet no data that may be of interest in the general problem of sex determination.

3. A Comparison between Protosexual and Eusexual Processes

If the theory of amphimixis is to be considered as the basic interpretation of sexuality (and there are as yet no strong objections against it) conjugation, transduction and even transformation are sexual processes.

If, however, we turn from such basic interpretation to the criterion of sex differentiation hardly any resemblance can be found between sex processes in bacteria and those occurring in eusexual organisms.

The formation of merozygotes and the unidirectional transfer of the genetic material in conjugation have already been cited as examples of basic differences with regard to the zygote formation. Nothing is known about the actual mechanisms of genic recombination and segregation in bacteria and therefore it is almost sure that they do not possess a classical type of meiosis.

One should not call the donor cells "male" and the recipient cells "female"

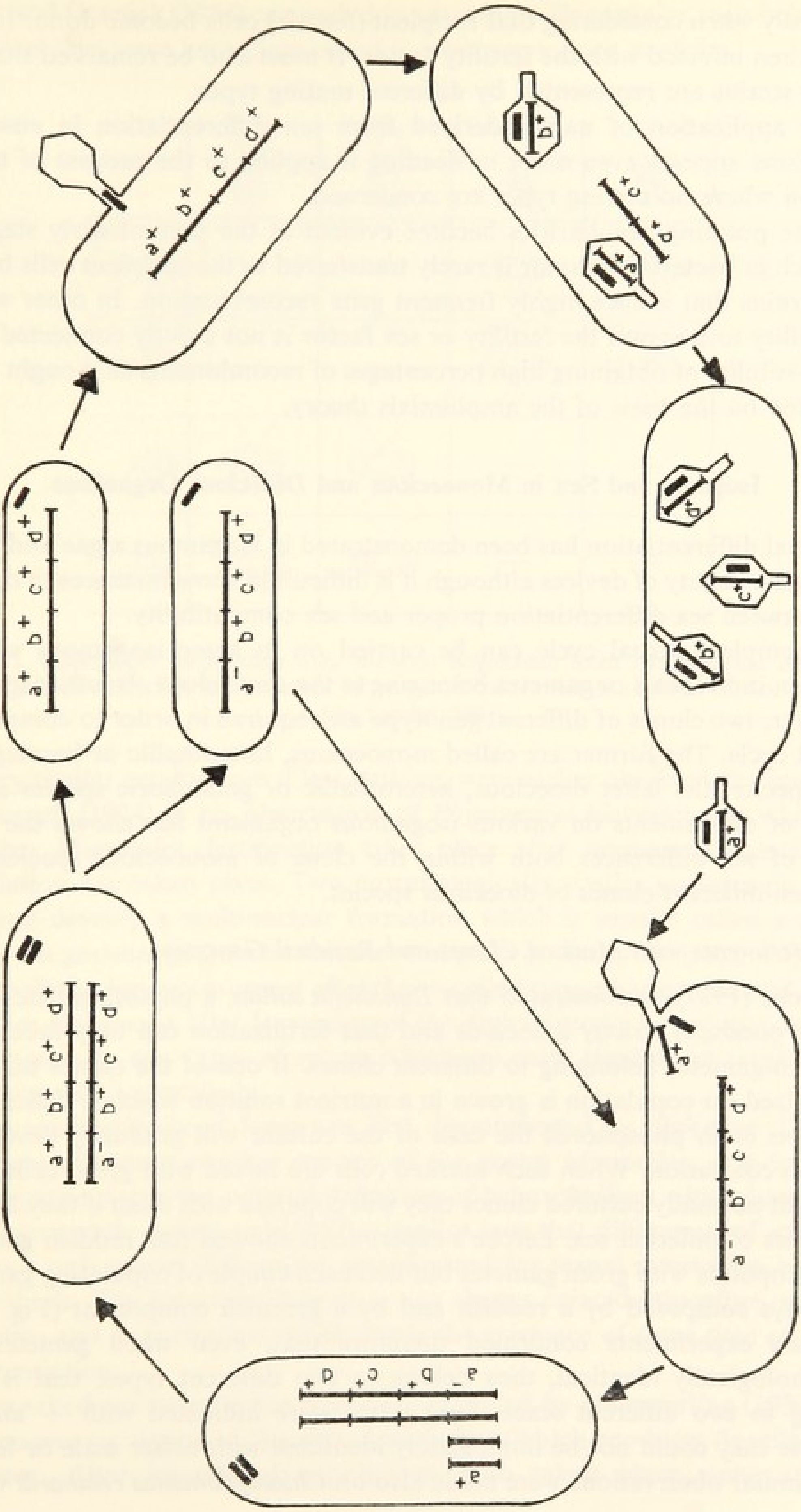

FIG. 3.2. A diagram of transduction. At the bottom: formation of mature phages, lysis of the donor bacterium and infection of recipient bacterium.

especially when considering that recipient (female) cells become donor (male) cells when infected with the fertility factor. It must also be remarked that the donor strains are represented by different mating types.

The application of names derived from sex differentiation in eusexual organisms appears even more misleading if applied to the process of transduction where no mating types are concerned.

Some puzzling peculiarities become evident at the present early stage of research in bacteria: F factor is rarely transferred to the recipient cells by the Hfr strains that induce highly frequent gene recombination. In other words the ability to transmit the fertility or sex factor is not strictly connected with the possibility of obtaining high percentages of recombinants as it ought to be expected on the basis of the amphimixis theory.

Isogamy and Sex in Monoecious and Dioecious Organisms

Sexual differentiation has been demonstrated in isogamous algae and fungi through a variety of devices although it is difficult in some instances to draw a line between sex differentiation proper and sex compatibility.

A complete sexual cycle can be carried on in some isogamous species between individuals or gametes belonging to the same clone. In other species, however, two clones of different genotype are required in order to complete a sexual cycle. The former are called monoecious, homothallic or hermaphrodite species, the latter dioecious, heterothallic or gonochoric species and a series of experiments on various isogamous organisms has shown the existence of sex differences both within the clone of monoecious species and between different clones of dioecious species.

1. Experiments with Marked Clones and Residual Gametes

Lerche (1937) demonstrated that *Dunaliella salina*, a phytoflagellate living in salt ponds, is strictly dioecious and that fertilization can only take place between gametes belonging to different clones. If one of the clones that will be utilized for copulation is grown in a nutrient solution which is deficient in nitrogen or in phosphorus the cells of the culture will gradually develop a reddish coloration. When such marked cells are mixed with green cells from different normally cultured clones they will copulate with them if they belong to clones of different sex. Lerche's experiments showed that reddish gametes never copulate with green gametes but that each couple of copulating gametes is always composed by a reddish and by a greenish component (Fig. 3.3). Lerche's experiments confirmed therefore that, even when gametes are morphologically identical, they belong to two different types, that is they belong to two different sexes. Such sexes were indicated with + and − because they could not be immediately identified with either male or female sex. Similar observations were made also on *Chlamydomonas reinhardi* where

Sager and Granick (1954) succeeded in marking with granules, cells belonging to clones that were kept illuminated in a nitrogen poor medium.

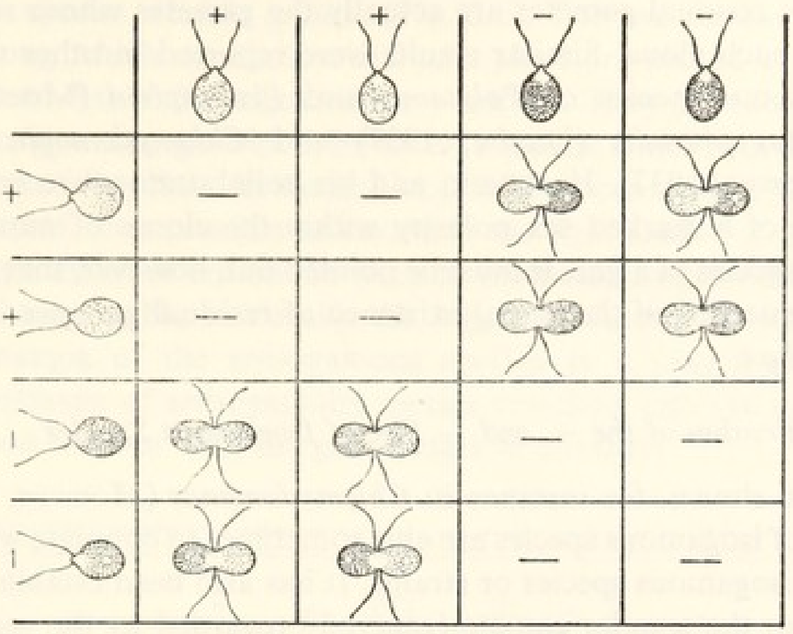

FIG. 3.3. Copulation takes place only between isogametes from two different dioecious clones, one of which has been marked by means of cultivation in a N and P poor medium (after Lerche, 1937).

Very similar results, even if less striking, were earlier obtained, for instance, by Burgeff (1924) in his observation of *Phycomyces blakesleyanus* where, as in other Mucorales, fertilization takes place after isogamous gametangial copulation has taken place. Two morphologically similar gametangia copulate and develop a multinuclear formation which is usually called a zygote where the gametangial nuclei form several pairs or dicaryons (see p. 35). They eventually undergo caryogamy after the so-called zygote has germinated. Meiosis takes place soon after formation of the diploid nuclei and gives rise in the sporangium to two types of spores which are quite similar but originate + or − mycelia respectively.

The sex bipolarity of fungi was first demonstrated by Blakeslee (1904) in *Rhizopus nigricans*, another species of the group Mucorales, and from his investigation arose the original definition of heterothallism which however—as Raper rightly points out (1957)—implies now that differences of any sort, and not only sexual differences, are required for sexual interaction between individuals. The heterothallism does not always coincide therefore with the dioecism and gonochorism which imply the existence of some type of sexual differentiation.

A marked sex polarity has been demonstrated by Hammerling (1934) also in monoecious strains of the alga *Acetabularia* which produces flagellate isogametes. After intraclonal copulations have taken place some residual gametes can be isolated from the zygotes and put into different containers where they remain quiescent until they are united with the residual gametes from different plants. It is possible to demonstrate through different combinations that the residual gametes are actually the gametes whose sex is supernumerary in each clone. Similar results were reported in other monoecious algae, like some species of *Polytoma* and *Protosiphon* (Moewus, 1935), *Haematococcus pluvialis* (Lerche, 1937) and *Chlamydomonas eugametos synoica* (Moewus, 1937). Hartmann and his collaborators are convinced of the existence of a marked sex polarity within the clones of monoecious or homothallic species of algae. It must be pointed out, however, that Pringsheim (1939) soon questioned the actual existence of residual gametes in *Polytoma* and *Protosiphon*.

2. The Identification of the + and − Sex of Isogamous Species

It has been shown, for instance in *Chlamydomonas* (Moewus, 1938), that the gametes of isogamous species are able sometimes to copulate with gametes of related anisogamous species or strains. It has also been established that in such instances the smaller gamete (generally regarded as the male gamete) always copulates with one of the two types of isogametes, and the bigger gamete with the other. The behaviour of + and − gametes toward the morphologically differentiated gametes therefore permits one to describe the + and − gametes of the isogamous species as male or female gametes according whether they copulate with the female or the male gamete of their anisogamous relatives (Fig. 3.4).

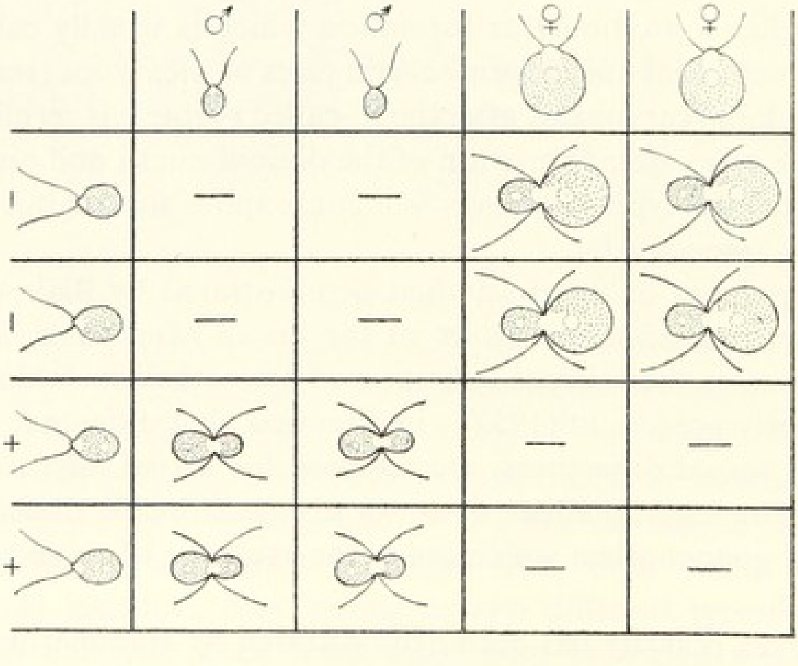

FIG. 3.4. Copulation of isogametes with anisogametes from different species enables to correlate sex (+ and −) of isogamous species with sex (♀ and ♂) of anisogamous species.

Such results, which were also obtained by Hartmann's school for a number of other algal species, had been already obtained by Blakeslee (1915) in his research on gametangial copulation between an heterothallic *Mucor* and the homothallic species *Absidia spinosa* (Fig. 3.5). The two classes of hyphae of *Mucor*, among which copulation can take place are placed at the opposite sides of a Petri dish and designated by + and −. They produce morphologically identical gametangia which, however, copulate either with the oogonia or with the antheridia of the homothallic *Absidia* whose hyphae are capable of self-fertilization. It is possible to correlate the significance of the + and − signs through different crosses between homothallic isogamous species. It is nevertheless impossible to classify the bigger as female and the smaller as male gametangia of the anisogamous species as it may happen that the bigger gametangia of anisogamous species copulate with the gametangia of an isogamous form or with the gametangia of another.

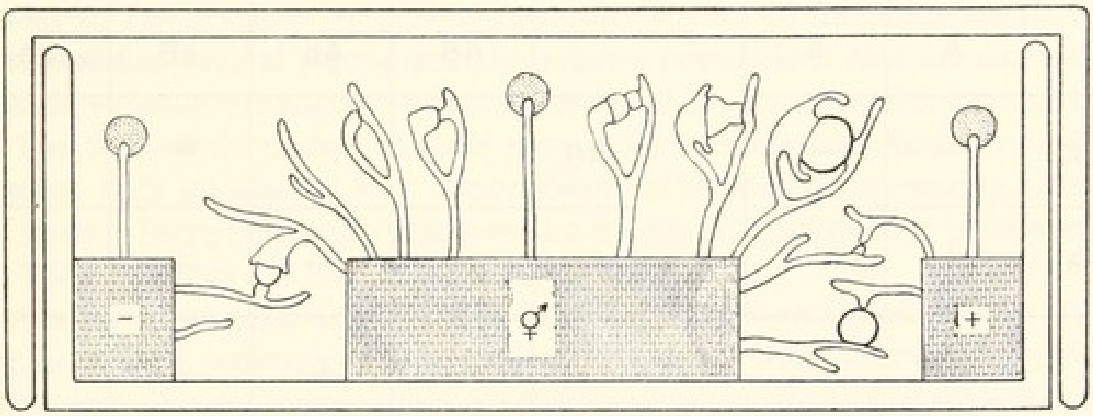

FIG. 3.5. Cross of the hermaphrodite *Absidia spinosa* with a dioecious *Mucor* (Blakeslee, 1915).

It appears therefore that relative size, which is important in distinguishing male from female gametes in higher organism, is not a sufficient criterion in sexing the Mucoraceae. The above experiments demonstrate nevertheless that a marked sex polarity exists in many isogamous forms and that in most cases they can be referred to the true sex differences shown by the higher organisms.

Incompatibility and the So-called Multipolar Sexuality

1. Bipolar and Tetrapolar Sexuality in Fungi

Self-pollination, and even pollination from certain other individuals, is prevented in a number of angiosperms, like *Nicotiana*, *Prunus*, *Veronica*, etc., by a series of alleles of one gene which are called incompatibility or sterility alleles and are commonly designated as S_1, S_2, S_3, etc. Pollen grains carrying a given incompatibility allele, are prevented from growing properly in the stylar tissue of a plant that carries the same allele. Regular cross breeding, or outbreeding, is thus secured in some hermaphrodite angiosperms by incompatibility factors.

Similar incompatibility factors cause heterothalliism in a number of haploid fungi where they favour outbreeding by greatly reducing the possibility of mating within breeding groups.

In certain species of fungi incompatibility factors may be represented at a single locus either by a single pair of alleles (Fig. 3.6) or by a large number of alleles, as in many species of basidiomycetes (Whitehouse, 1949). This pattern of mating type determination has often been termed bipolar sexuality although, as pointed out by Raper (1952), some of the heterothallic species showing this peculiar pattern are devoid of differentiated sex organs and any cell of the thallus is potentially capable of mating.

	A	a
A	—	+
a	+	—

	AB	Ab	aB	ab
AB	—	—	—	+
Ab	—	—	+	—
aB	—	+	—	—
ab	+	—	—	—

FIG. 3.6. Incompatibility in fungi determined by a single (left) and by two (right) series of allelomorphs.

Kniep first described in some Basidiomycetes two series of allelomorphs for incompatibility (Fig. 3.7). As many as twenty-seven alleles have been described at each locus although Papazian (1951) demonstrated that numerous pseudoalleles for incompatibility, which are located at closely linked loci, act as a physiological unit in *Schizophyllum commune*. The same probably happens in other series of incompatibility factors. The peculiar type of heterothallism which is determined by two series of incompatibility alleles has been named tetrapolar sexuality although such multiple alleles can hardly be regarded as sex genes because incompatibility, being the result of allelic identity, can be regarded the contrary of sex differentiation.

Incompatibility genes, however, cannot easily be distinguished in practice from sex genes and they partly carry on, by reducing inbreeding, one of the main function of the sex genes which consists in promoting genetic variability. Figure 3.6 shows that inbreeding is indeed reduced to a half in the bipolar and to a quarter in the tetrapolar forms of fungi.

An allelic series of incompatibility factors has been demonstrated also in morphologically and functionally hermaphrodite strains of ascomycetes (Dodge, 1932), including *Neurospora.* The similarity with incompatibility systems of angiosperms is particularly striking in such cases where the action of sex genes is demonstrated by the differentiation within each strain of antheridia and ascogonia, or of spermatia and ascogonia. Fusion of such elements can, however, take place only with the complementary and opposite sex elements of compatible strains.

A situation which at first sight appears similar is found in Infusoria.

2. *Mating Types and Sex in Infusoria*

Genetical investigations have shown that *Paramecium aurelia, Paramecium bursaria, Tetrahymena pyriformis* and other species of Infusoria are actually composed of several reproductively isolated populations that are commonly named varieties or syngens and constitute to all intents, natural cryptic species.

The taxonomic entity which is named *Paramecium aurelia* is formed by sixteen such syngens at least (Sonneborn, 1957) and an increasing number of them is being constantly discovered not only in *P. aurelia* but in all species of Infusoria that are being sufficiently investigated.

Mating types were discovered by Sonneborn in *Paramecium aurelia* (1937) by artificially separating the exconjugants which were afterwards cultured separately. No conjugation was observed within the clones that were obtained from each exconjugant but mass conjugation took place when the clones from the original pair were brought together.

The term mating type is now being more or less loosely employed in organisms other than Infusoria when members of a given clone or strain never mate with each other and only mate with members of certain different strains.

Thus the mating type has been considered something equivalent to sex. It must be remembered, however, that conjugation of Infusoria can be regarded as the mating of two hermaphrodite gamonts because the migrating and the standing pronuclei have been assimilated to a male and a female gamete respectively. The occurrence of autogamy in *Paramecium* shows on the other hand that the mating type system cannot be assimilated to the self-sterility systems existing in higher plants.

The varieties or syngens belonging to each morphologically defined species (*Paramecium aurelia, Euplotes patella* or *Tetrahymena pyriformis*), are marked with arabic numerals and the mating types with roman numerals. Each syngen of *Paramecium aurelia* is composed by two mating types so that mating types I and II belong to syngen 1 and mating types XI and XII to syngen 6 but it is not always so in other species: four mating types are known in syngen I, eight in syngen II of *Paramecium bursaria*, nine in one "variety" of *Tetrahymena pyriformis* (Elliot, 1959). It is evident therefore that mating types of many species of Infusoria have nothing to do with sex differentiation of higher organisms even from the point of view of a double polarity.

The study of mating type determination showed that this peculiar reproductive system is largely dependent upon the constitution of the macronuclei and Sonneborn introduced the notion of caryonide in order to indicate Infusoria that contain macronuclei derived from a single macronucleus. One clone may actually be composed of two or more caryonides because the exconjugants occasionally develop more than two macronuclei.

It was early established (Sonneborn, 1939) that inheritance of mating types I and II of *Paramecium aurelia* depends on two allelic genes *A* and *a* so that members of certain caryonides derived from wild individuals were all of like mating type and members of other caryonides were of two complementary mating types.

Syngens of *Paramecium aurelia* have subsequently been grouped into two classes. In syngens belonging to group A (as in syngens of *Paramecium bursaria*) mating type determination takes place through the action of nuclear genes in the way described above. In syngens belonging to type B (syngens 2, 4, 5, 8,) the cytoplasm plays a very important role in determining mating types as it has been demonstrated that individuals with the same genotype show different mating types when they have inherited a different cytoplasm. If mating types VII and VIII of syngen 4 are taken into consideration, it can be shown that, after conjugation, individuals developed from mating type VII will belong to mating type VII and individuals developed from the exconjugant belonging to mating type VIII belong to mating type VIII even when micronuclei are identical. Delicate nucleo cytoplasmic interactions take place, however, in group B and it has been supposed (Nanney, 1953) that mating type differences are ultimately determined in both groups by gene dosage differences in the macronuclei of cells of different types. The macronucleus previously occupying the cell produces, in Nanney's interpretation, a cytoplasmic substance which is responsible for the achievement of the higher dosage levels. The different types of macronuclei determine only different mating types on the different caryonides of the Group A syngens, but they determine both different mating type and different amounts of the cytoplasmic agent in the Group B syngens.

It could not be decided whether the cytoplasmic agent is a self reproducing substance or a substance directly produced by the nucleus: when cytoplasmic fusion was induced in conjugating pairs of type VII and VIII a change from type VII to type VIII took place (Nanney, 1957). Unstable caryonides, in which selfing occurred, showed on the other hand a change in the reverse direction and exposure to higher than normal temperatures increased the amount of changes.

Research in Infusoria has unfortunately (for the mating type problem!) concentrated lately in such fascinating problems as the killer trait inheritance or in the genetics of the H serotype systems (for which no correlation could be established, according to Nanney, 1960, with the mating systems) and many aspects of mating type determination are not clear yet.

The mating type system stands, however, as an outstanding example of the complexity of genetic systems in such "lower" organisms like Infusoria and of the multi-sided aspects of the sexual reproductive systems.

Relative Sexuality

The notion of relative sexuality has been very popular among biologists in the early thirties when it appeared as the most elementary expression of sexual differentiation. The growing interest in the problems of mating type determination, the developments of research on sex in bacteria and the failure in confirming alleged mechanisms of relative sexuality in *Chlamydomonas* have lately contributed to put the problems of relative sexuality in the background.

Hartmann gave the first demonstration of relative sexuality in his work on *Ectocarpus siliculosus* from the Bay of Naples (1925). The gametes of the 160 gametophytes which were investigated showed a marked female and male differentiation but 1018 gamete combinations showed markedly different results. Some combinations resulted in a rapid fusion of the gametes and in the formation of very numerous zygotes, some gave less numerous zygotes after what can be considered a slow process of fertilization, and a few other combinations produced a few zygotes only. Hartmann suggested the existence of strong, medium and weak male gametes, of strong, medium and weak female gametes according to the velocity of the fertilization processes and to the number of zygotes formed in their different combinations.

The most interesting result in Hartmann's experiments was that fertilization took place in a few instances (less than 3 per cent of the total combinations) between gametes of the same sex but of different strengths. In other words zygotes can either be the result of fertilization between male and female gametes or between weak male and strong male, between weak female and strong female gametes. The sexual behaviour of the copulating gametes from plants of the same sex corresponds therefore in some combinations to the sexual behaviour of the gametes of the opposite sex.

Hartmann concluded therefore that the gametes of *Ectocarpus siliculosus* show only relative sexual differentiation, that is they are not absolutely male or female gametes. The above mentioned differences in the strength of one sex may be such that a normally female gamete may function as a female towards a male gamete and as a male toward a stronger female gamete.

The first results in *Ectocarpus* were confirmed in 1934 and in 1937 and similar instances of relative sexuality were also discovered in two species of green algae *Enteromorpha granulosa* and *Bryopsis mucosa* and it has been reported also for a species of *Spirogyra*.

Hartmann's research on relative sexuality has demonstrated, therefore, the existence of a sexual bipotency in haploids. They do not differ in this respect from diploid organisms like *Drosophila* and *Lymantria* where the genes for both sexes are present in each sex genotype (see Chapter 5). In Hartmann's interpretation relative sexuality is due on the other hand to hypothetical female and male potencies that are different from the sex genes or sex realizators, which are located on the chromosomes (see p. 69).

Both the notions of sex polarity in isogamous species and of relative sexuality have been questioned in Algae (Mainx, 1933; Pringsheim, 1939), where differences in ripeness seem to explain in some instances alleged differences in the polarity of the gametes. Relative sexuality has, however, been ascertained in several heterothallic phycomycetes, or water moulds (Couch, 1926; Bishop, 1940; Raper, 1947).

Raper (1947) describes the geographic variations in the mating patterns of strains of *Achlya bisexualis* from England and from Northern Illinois. Six sexual strains from Illinois were each self-sterile but cross-fertile in all combinations and the author was able to place them linearly, with respect to male and female sex potentialities, in such a way that each strain reacted as a male to those on its right and as a female to those on the left. When such strains were mated to one strain collected in England they all reacted as females. In this way each sexual strain reacts as a male or as a female in accordance with its positions in the series, with the exception of the two terminal strains which represent the strong male and female strains.

When a male and a female mycelium are put on a semi-solid medium in the same dish, zygotes are formed along the boundaries between the spreading mycelia. The stronger the difference in sex polarity, the stronger is the production of zygotes between the two strains, as indicated by the marked lines of zygotes that are formed between the mycelia (Fig. 3.7). The similarity with the Hartmann's findings in *Ectocarpus* is thus evident.

The work of Couch on *Dichtyuchus monosporus* provided in 1926 the first and only available data to solve the problem of relative sexuality. Crosses between pure male and female strains produced intermediate hermaphrodites of various grades, neuters and also pure male and female strains. In other words a continuous and graded series of sex phenotypes between pure male and female strains was obtained. An analysis of such results indicates therefore that multiple sex genotypes have arisen through random segregation of sex factors during the meiotic processes in the heterozygous oospore. This interpretation requires further investigation but it appears highly probable from the available data that the various sex phenotypes occurring in the known instances of relative sexuality are the expressions of different equilibria between multiple genes for male and female sex in the haploid phase. The alternative interpretation of single sex genes or realizators of different strengths appears less probable in most instances.

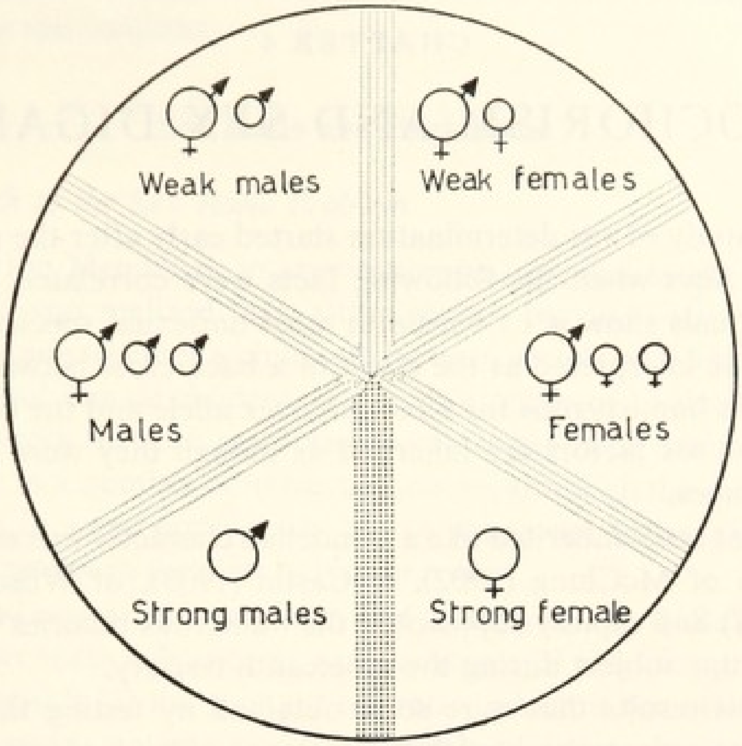

FIG. 3.7. Zygote formation between mycelia of different sex intensity. High zygote production is obtained between strong male and female mycelia, low production between weak males and females.

It appears convenient therefore to consider relative sexuality as a sort of unbalanced hermaphroditism (see Chapter 7) in the haplophase (Fig. 3.7) where the two terminal strains represent pure males and females and the intergrade phenotypes represent graded potential hermaphrodites which are more or less oriented toward male or female reactions.

The types of sex determination seem to be essentially alike in relative sexuality and in unbalanced hermaphroditism.

CHAPTER 4

GONOCHORISM AND SEX DIGAMETY

THE modern study of sex determination started early after the rediscovery of the Mendel's laws when the following facts were correlated: (1) male and female individuals show a 1 : 1 ratio in most unisexual species; (2) the 1 : 1 sex ratio can be interpreted as the result of a back-cross between individuals one of which is homozygous for a recessive sex allele and the other is heterozygous; (3) the sex factors are inherited as though they were located in the sex chromosomes.

The idea that sex is inherited like a mendelian character was early supported by the works of McClung (1902), of Castle (1903), of Wilson (1905) and Correns (1907) and rapidly supplanted the numerous theories that had been advanced on this subject during the nineteenth century.

The brilliant results that were soon obtained by testing this early interpretation led to overemphasis of the importance of the back-cross mechanism in the evolution of sex determination and to the establishment of two great opposed categories of sex determination (Hartmann, 1943, 1956): the genotypic sex determination, which takes place in unisexual organisms whose sex is determined by a sex chromosome mechanism, and the phenotypic (or modificatory) sex determination, which takes place in all hermaphrodite plants and animals and also in the unisexual organisms whose sex appears to be mainly influenced by environmental factors. Hereditary determination, practically coinciding with sex digamety, was thus opposed to what has been considered a non-hereditary sex determination, a view which appears to be still accepted by the authors of many treatises when mentioning for instance, the experiments on sex determination in *Bonellia* or in *Ophryotrocha*.

It is still very difficult to grasp the real genetic situations prevailing in the mixed category of the so-called phenotypic determination and to understand the relationships existing between sex determining mechanisms in hermaphrodites and in truly unisexual organisms. This is mainly due to the fact that the evolutionary connexions linking sex chromosomes and autosomes are still very little known and on the other hand significant genetical work on sex in hermaphrodites and in labile gonochorists is still very scarce.

The most advanced work on plants and animals that show sex digamety has nevertheless given clear indications that the mechanism discovered by the early students of sex determination, although it is highly specialized, is by no means isolated in the evolution of the sex determining systems. The mechanism of the sex balance, discovered in *Drosophila melanogaster*, gave the demonstration that the early mendelian interpretation has a purely formal value and provided at the same time the tools for a general interpretation of sex determining mechanisms.

Sex Ratio and Back-cross

1. The Approach to the Sex Ratio Problem

Long before the Mendelian interpretation was applied to the study of sex determination it was realized that, although it is beyond our possibilities of prediction to decide whether a child will be born a male or a female, the ratio between male and female children in a high number of births is well established and it ranges between restricted limits. It has been demonstrated that the same is true in a number of unisexual species although the sex ratio shows wide variations in some populations where it appears to be adapted to the need of reproduction in peculiar environments.

The numerical relationship of males and females can be shifted by different causes that exert their influence at various stages of the life cycle. We must therefore distinguish (1) the primary sex ratio, which is the ratio at the time of fertilization and is not practically accessible to direct observation, (2) the secondary sex ratio or the sex ratio at birth and (3) the tertiary sex ratio in adult individuals when, according to most authors, the period of sexual maturity is reached.

Sex ratio is expressed either as number of males per 100 or per 1000 females or as the percentage of males in the total population.

The different individuals should be sexed as early as possible in order to determine with accuracy the primary sex ratio which is supposed to be the nearest to the mendelian sex ratio, because a selective mortality has been demonstrated in most species, both in the very early embryonic stages, and after sex differentiation has become evident.

The secondary sex ratio among the United States whites is approximately 106 male to 100 female babies, among the negroes there are 102·6 boys to 100 girls. In Italy there are approximately 105 males for 100 females at birth and the secondary sex ratio varies in different countries and in different racial groups within the same country. Still it is always significantly different from the 1 : 1 ratio.

It must be mentioned that among children there is a differential mortality so that at sex maturity a ratio of 1 : 1 is established.

The sex ratio of aborted embryos and stillborn children shows also a greater mortality of males than of females and for this reason the primary sex ratio in man has been generally estimated as 125 to 135 male to each 100 female zygotes although some estimates have gone as high as 170 males to 100 female conceptions and others as low as about 107 males to 100 females.

McKeown and Lowe (1951) contended on the other hand that the sex ratio of live embryos is about 50 per cent in the seventh month although a very high percentage of abortions (at least one half) take place in the first 3 months of pregnancy. Colombo (1957) excluded a strong differential mortality because he did not find significant differences in the secondary sex ratios when he divided nearly ten thousand women into classes with high and low abortion rates.

It must be concluded in any case that the primary sex ratio is significantly different from the expected 1 : 1 ratio in man. It has been suggested that the male determining sperm—which carries the Y chromosome— is more motile than the X carrying sperm or that the environment of the female ducts is more favourable to the survival of X than of Y sperms. There might be a preferential or selective fertilization due to a more ready reaction of the eggs to the approach of the male determining than of the female determining sperm. Dahlberg (1951) advanced the hypothesis that over-ripe eggs are preferentially fertilized by male determining sperms and for the same reason the male embryos are subject to a high relative mortality.

A higher mortality of males as compared to females has been demonstrated not only in man but also in other mammals. Lindahl and Sundell (1958) were able to sex embryos of the golden hamster before uterine implantation and they found at this early stage a very high sex ratio with a sex ratio at birth equal to one half. Such high mortality has been explained as the result of a very intense metabolism that is established after sex differentiation and makes male individuals less resistant to unfavourable conditions. In the case of males belonging to species with a single X chromosome it is also supposed that males are constitutionally weaker than females for the presence of subvital mutations on the single X chromosome that cannot be masked, as in the female sex, by their dominant alleles in the second X chromosome (Crew, 1937).

Although man is undoubtedly the species where sex ratio has been analysed most thoroughly it can be stated that also in most species there is established a secondary sex ratio which is very near to 1 and we can consider equality as the general expression of the phenomenon (Pelseneer, 1926). When minor variations are ascertained they can be explained with influences of secondary factors.

A special case is presented by animal populations where the sex ratio approaches, on the whole, the 1 : 1 proportion but the single families, which compose the population, show either marked tendency toward the production of male or to the production of female individuals (see Chapter 6).

An interesting question concerning sex ratio in man arose when it was found that in war times the ratio of males is higher than during peacetime.

A recent explanation of the phenomenon relates the change in sex ratio to early fertile as compared to late fertile marriages (Bernstein, 1958): when the first child is born within the first 18 months of marriage the sex ratio of the first born is 124 : 100, but when the first child is born later the ratio falls to 99 : 100. Early fertile couples appear to be more frequent in war-time than in peacetime and therefore they contribute a high proportion of male sons to the population. Savorgnan (1921) advanced the interpretation that the prolonged interval between successive deliveries in war-time reduces the number of abortions and consequently raises the proportion of males at birth by approaching the primary sex ratio.

Some peculiarities of the sex ratios can thus be explained through the influence of environmental factors: selective influence of the female ducts on the X carrying sperms which shifts the primary sex ratio or selective mortality of the male embryos which influences secondary sex ratio. It has been also established that selective mortality of the males approaches in man the 1 : 1 ratio at the time of sexual maturity. This is an important condition not only in man but in all the species which rear their young because, as Fisher (1930) pointed out, it allows subdivision of the efforts required to raise the offspring.

It appears theoretically possible to control sex ratios in animals by artificial means. Some efforts have already been made in this direction especially by trying to select male or female determining sperms outside the body of experimental animals. Treatment of bull spermatazoa with counter streaming centrifugation (Lindhal, 1958) indicated that the female determining spermatozoa are more sensitive to mechanical stress than the Y bearing spermatozoa and therefore a decreased fertility occurs with a higher proportion of males.

Concordant results by Shreder (1934) and by Gordon (1957) on sperms of rabbits indicate that passing of electric current through a sperm suspension causes one type of sperm to pass to one pole and the other preferably to the opposite pole.

Nevo, Michaeli and Schindler have repeated such experiments with bull and rabbit spermatozoa (Rothschild, 1960) and they have found that all spermatozoa migrate toward the anode in an electric field because, at normal pH, they have negative charges on their surfaces.

A report by Shettles (1960) claiming that there is a sharp difference in the size and form male and female producing types of human spermatozoa has also been criticized (Rothschild, 1960; Bishop, 1961).

The above experiments and observations gave apparent support to simple explanations of sex ratios variations and at the same time gave hope for a near solution of the problem of the environmental control of the sex of the progeny. It must be acknowledged, however, that their results are subject at present to much perplexity.

Experiments by Weir (1958, 1962) on mice belonging to two different strains, one with a high male ratio, another with a low male ratio showed that only the male parents are responsible for the sex ratio of the litter. It is also highly probable that differences in the secondary sex ratios are correlated with similar sex ratios at conception. It has been attempted (Haubenstock, 1961) to influence the sex ratios by inducing changes in blood pH because the males that induce a high male ratio in the progeny belong, in Weir's experiments, to strains selected for a high blood pH but no correlated changes in the sex ratio of the offspring have been produced.

Experiments on mice show therefore the existence of genetic mechanisms that in each strain exert their influence on sex ratio.

2. The Back-cross Mechanism in the Bryonia *Experiments and the Early Genetic Sexual Formulae*

Mendelian genetics established that the back-cross of the F_1 heterozygote with the recessive parent results in a ratio of one dominant phenotype to one recessive phenotype. It was therefore supposed that the two sexes form a couple of two allelic characters and that one sex is homozygous (homogametic sex) and the other is heterozygous (digametic sex) with reference to sex genes.

The first genetic demonstration of this hypothesis was given by Correns (1907) with his crosses of two species of flowering plants belonging to the genus *Bryonia. Bryonia dioica* is unisexual and *Bryonia alba* is hermaphrodite and the following results were obtained using the two species alternatively as pollen producers (♂) or as egg producers (♀):

(a) *Bryonia dioica* ♀ × *B.alba* ♂*:* the progeny is composed almost exclusively by female hybrids (587 ♀♀ and 2 ♂♂);

(b) *Bryonia alba* ♀ × *B.dioica* ♂*:* the progeny of 358 hybrid plants is composed by 171 purely male and by 187 female plants.

All F_1 hybrids resulting were sterile. Correns drew the conclusion from the (a) experiment that the female plants of *Bryonia dioica* produce gametes of only one type and deduced from the (b) experiment that the male plants produce two types of gametes, male and female determining, in approximately equal proportions. The character of dioecism was also dominant upon the character of monoecism. Therefore the male sex is dominant and heterozygous, the female sex is homozygous and the identity of the type of sex inheritance in *Bryonia* and the back-cross mechanism is formally perfect.

It was also concluded on the basis of Correns' experiments that male and female gametes of the monoecious *Bryonia alba* have identical genetic constitutions regarding sex determining factors, a conclusion that was later unduly extended to all other hermaphrodite organisms.

The experiments on *Bryonia* have been recently confirmed and extended by Heilbronn (1942-53) who obtained fertile hybrids that could be back-crossed to *Bryonia alba*. Essentially similar results were also obtained by Murray

(1940) in crosses between dioecious species of *Acnida* and monoecious *Amaranthus*.

Correns derived from the *Bryonia* experiments the formulas FF = ♀ and FM = ♂, with M epistatic, where F indicates the female and M the male sex determiners or genes. As this simple formulation could not interpret the occasional appearance of male characters in female individuals, the existence of a AG complex was postulated, with A indicating the male and G the female potencies which, in Hartmann's interpretation (1930) are present both in male and in female individuals.

The F and M sex factors, determiners or genes were supposed to decide whether the A or the G potency would become more active in each individual and were called realizators in v. Wettstein terminology (1924b). For this reason sex determining genes are often called sex realizators especially by German authors. The formulas suggested for sex determination in flowering plants were the following:

$$(A\cancel{G})\ \mathrm{MF} = \text{♂} \text{ and } (\cancel{A}G)\ \mathrm{FF} = \text{♀}$$

The lines barring G in the male sex formula and A in the female sex formula indicate the inhibiting effects of the male and of the female realizators upon the female (G) and the male (A) potencies respectively.

A quite different and simpler formulation was advanced on the basis of Bridges experiments on *Drosophila* (Chapter 5) but the supposed existence of a double system of sex determination formed by the AG complex and by the sex realizators has until recently been a matter of lively discussion (Goldschmidt, 1955; Hartmann, 1956; Wiese, 1960).

The Heteromorphic Sex Chromosomes

Before Correns could provide the genetic demonstration of the female homozygosis and male heterozygosis in *Bryonia dioica*, cytologists discovered the existence of chromosomes that were different from one sex to another. They are now named sex chromosomes, heterochromosomes, X or Y chromosomes, and during meiosis they are unequally distributed in the gametes of the two sexes.

The inheritance of the heterochromosomes thus visibly marks the digamety of one of the sexes (Fig. 4.1) although it must be mentioned that in many species (in *Bryonia dioica* for instance) sex digamety, although genetically well established, is not expressed by the presence of morphologically detectable, or heteromorphic, sex chromosomes. Sex chromosomes appeared therefore to be both sex and sex ratio determining. It must be pointed out, however, that sex ratio can be under the control of environmental or autosomic factors or both even in species where, as in aphids for instance, well developed sex chromosomes are shown.

In his early work on grasshoppers McClung (1902) assumed the X bearing class of sperms to be male producing while the no X class of sperm is female producing. After a few years of contrasting views, due to the false idea that females are devoid of sex chromosomes, the confusion was dispelled by Wilson's demonstration (1905, 1906) that the female possesses one more chromosome than the male in what, following Wilson's work, is now called the *Protenor* type of male digamety.

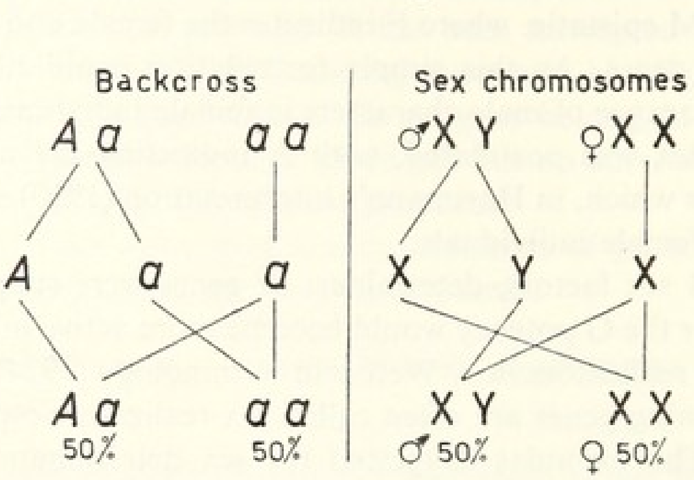

FIG. 4.1. Comparison between the transmission of genes in back-cross and the transmission of sex chromosomes in a species showing male digamety.

We may now follow the Wilson's scheme (1928) in illustrating the simplest caryological mechanisms of sex digamety.

1. The Lygaeus *Type of Male Digamety*

This type is named from an hemipteran insect, *Lygaeus turcicus*, which was studied by Wilson in his basic work of 1906 (Fig. 4.2). The females have

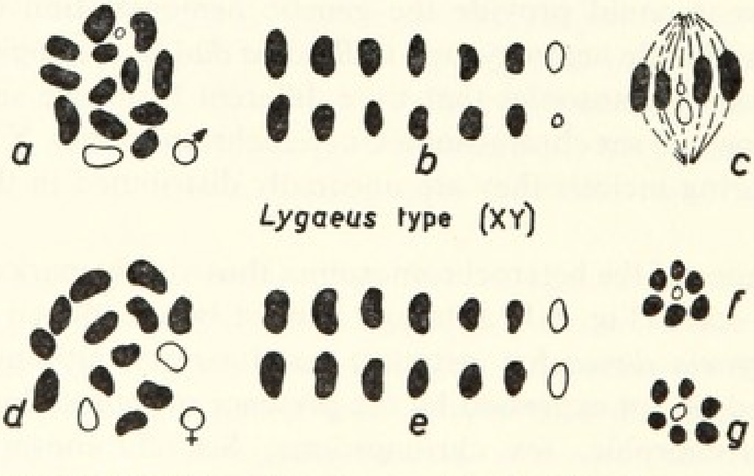

FIG. 4.2. Autosome (solid) and sex chromosomes (outlined) in *Lygaeus turcicus*: a and d, diploid metaphases from a male and a female individual: b and e, series of homologous pairs of chromosomes from metaphases; c, the second spermatogenetic division with Y separating from X (below); f and g, two haploid sets of future spermatids, one (f) containing the Y and the other (g) the X chromosome (Wilson, 1906).

twelve autosomes and two X chromosomes, the males one X chromosome and a smaller Y heterochromosome. The two X chromosomes of the females pair at meiosis, migrate to opposite poles of the achromatic spindle and in this way all the female gametes receive an X chromosome. In the male sex, after a partial pairing of X and Y, each of them goes to a different pole so that one half of the gametes will receive six autosomes and the X chromosome (6 A + X) and the other half receives the same haploid set of autosomes and the Y chromosome (6 A + Y). Egg fertilization with the X bearing sperm will develop a female zygote (12 A + XX) and fertilization with the other sperm type will give rise to a male zygote (12 A + XY). The homogametic and the digametic sexes have the same number of chromosomes in the *Lygaeus* type and only the shape and the structure of the Y chromosome are different from those of the X chromosomes. This makes the two categories of male gametes cytologically identifiable.

The fruit fly, *Drosophila melanogaster*, belongs to the *Lygaeus* type. The female has three pairs of autosomes and one pair of rod-like X chromosomes. In the male there is only the rod-like X chromosome, the other member of the pair being represented by a Y chromosome with a hook shaped or bent end. Females have thus the chromosome constitution 6 A + XX and males 6 A + XY.

It has been ascertained that in man normal males possess both X and Y chromosomes and therefore they produce X or Y bearing sperm the same as a number of nematode, insect and mammalian species, which have visible X and Y chromosomes.

The first example of sex chromosome in plants has been discovered in the liverwort *Sphaerocarpus* where a very large X chromosome and a much smaller Y chromosome have been discovered (Allen, 1917). A similar situation exists among Angiospermae in *Melandrium*, first studied by Winge and by Blackburn (1923), where the Y is bigger than the X chromosome.

In some species differences between the X and the Y chromosomes are not detectable by differences in shape or size but the XY pair may still be recognized by its behaviour as in the case of the hemipteran *Oncopeltus fasciatus* where the X and Y chromosomes precede autosomes during the anaphase migration (Wilson, 1909–12). Finally in many species both of plants and animals the sexual digamety of the male sex can only be demonstrated by genetical methods since the X and Y chromosomes cannot be identified cytologically.

2. The Protenor *Type of Male Digamety*

The first researches by McClung on grasshoppers referred to a situation where the female homozygous sex has two X chromosomes and the male sex one X and no Y chromosome.

In *Protenor belfragei*, Wilson demonstrated that the female has fourteen chromosomes (12 A + XX) and the male only thirteen chromosomes (12 A + X) (Fig. 4.3). Females have thus one chromosome more than males, a situation which is supposed to have evolved from increasing reduction of the Y chromosome and that is expressed by the general heterochromosomal formulae XO (♂): XX (♀).

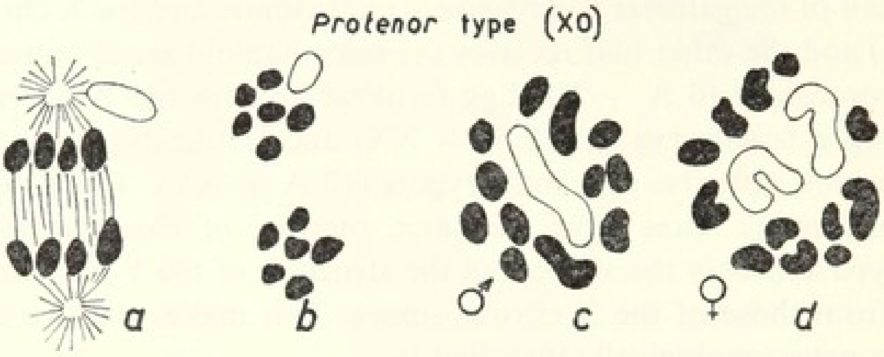

FIG. 4.3. Chromosomes of *Protenor belfragei*: a, anaphase of the second spermatogenetic division; b, two haploid sets of future spermatids, one (above) containing the X and the other (below) without sex chromosome; c and d, diploid spermatogonial and oogonial metaphases (Wilson, 1906).

Species of animals that follow the *Protenor* scheme are found in several classes and even within the same genus species following the two types of digamety are found as for instance *Drosophila melanogaster* (XY) and *Drosophila orbospiracula*, *longala*, *mercatorum*, *annulimana* that have become XO in the male sex (Patterson and Stone, 1952).

It is evident that the differences between the two categories of male gametes are most evident in the animals of the XO type. Mülsow (1912a) described a very interesting case of digamety in the sperms of the nematode *Ancyrocanthus cystidicola* where chromosomes (distinguishable in the ripe sperms) are often united in quartets developed from a single spermatocyte: one half of them shows six and the other half five chromosomes (Fig. 4.4) and all the eggs show six chromosomes.

It has been shown (see Chapter 6) that the multiple sex chromosomes

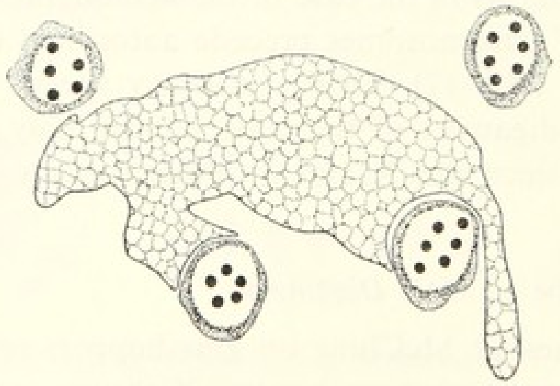

FIG. 4.4. The four products of meiosis remain united in the spermatogenesis of the nematode *Ancyracanthus cystidicola* : two cells carry six and two only five chromosomes (Mülsow, 1912).

mechanism has evolved in most cases from cytological situations of the *Protenor* type.

3. Sex Chromosomes in Female Digamety

Some copepods and isopods among crustaceans, Lepidoptera and Chrysopidae among the insects, some fish, amphibian and reptilian species and birds among vertebrates, are homogametic in the male and digametic in the female sex.

Fragaria orientalis is the only plant species known to have digametic female sex and heteromorphic sex chromosomes (Kihara, 1930).

The formulation ZW : ZZ instead of XY : XX is often used, for exclusively practical reasons, in such instances where the function of the sex chromosome seem to be reversed. Natural populations and aquarium strains showing female or male digamety have been found nevertheless within the same species of fishes (see Chapter 6). Male and female digamety cannot be considered therefore as two substantially different kinds of sex determination although some authors find it expedient to use different symbols to indicate sex chromosomes for male and for female digametic individuals.

The study of sex chromosomes has proved difficult in Lepidoptera: Seiler in 1914 demonstrated that in the moth *Phragmatobia fuliginosa* females show a digamety of the *Lygaeus* type. Females of the moth *Talaeporia tubulosa* have fifty-nine chromosomes (58 A + X) and males sixty chromosomes (58 A + XX) and therefore both the XY and the XO types have been demonstrated in female digamety. Chrysopidae also show female digamety (Hichijo, 1943).

Recent research on embryonic cells lead first to identify the X (=Z) chromosomes in a number of bird species (Van Brink, 1959; Ohno, 1961) and later both the X and the Y chromosomes have been found (Rothfels *et al.*, 1963) in the common budgerigar *Melopsittacus undulatus*, which has therefore a XY (—WZ) constitution in the female sex.

Improvements in cytological techniques have made it possible to ascertain the existence of X and Y chromosomes in two species of female snakes (Beçak, 1962) although previous claims that female digamety could be cytologically detected in reptiles (Oguma, 1934) has been criticized in a fundamental work by Matthey (1949) and also by other authors.

Female digamety has even been cytologically established in the frog *Xenopus laevis* (Weiler and Ohno, 1962).

Cytological works by Beermann (1954) and by Ruesch (1958) have shown the existence of female digamety with heteromorphic sex chromosomes in two species of Cyclopidae copepods. Cytological observation on female digamety in isopods are based so far only on a work by Staiger and Bocquet (1954) that have found multiple sex chromosomes in female individuals of *Jaera marina*.

Sex-linked Inheritance

It has been shown that the X (or Z) chromosome of the digametic sex is inherited from the parent of homogametic sex: in case of male digamety the X chromosome is inherited from the female and in case of female digamety it is inherited from the male parent. The Y (or W) chromosome is transmitted only from father to son in case of male digamety or directly from mother to daughter in the known instances of female digamety.

Doncaster (1907) and Morgan (1910) discovered some genes that follow the X or the Y chromosomes in their transmission and that are now called sex-linked genes. They are not necessarily concerned with sex characters. The discovery that their peculiar type of inheritance is due to the fact that they are located on the sex chromosomes has represented at the same time one of the earliest proofs for the theory of mendelian sex determination and the beginning of modern genetic.

1. *Sex-Linked Genes in* Drosophila (*Male Digamety*)

Morgan studied crosses between the red-eyed wild type and the white-eyed mutant of *Drosophila melanogaster*. The couple of alleles that are responsible for the two colorations are indicated in Fig. 4.5 with W (red eyes) and w (white eyes).

The cross of a white-eyed male with an homozygous red-eyed female produces an offspring (F_1) of red-eyed individuals only. In the F_2 generation, however, all the females have red eyes but half of the males have red and the other half white eyes.

The reciprocal cross of a red-eyed male with a white-eyed female produces white-eyed males and red-eyed females and the F_2 generation is composed of

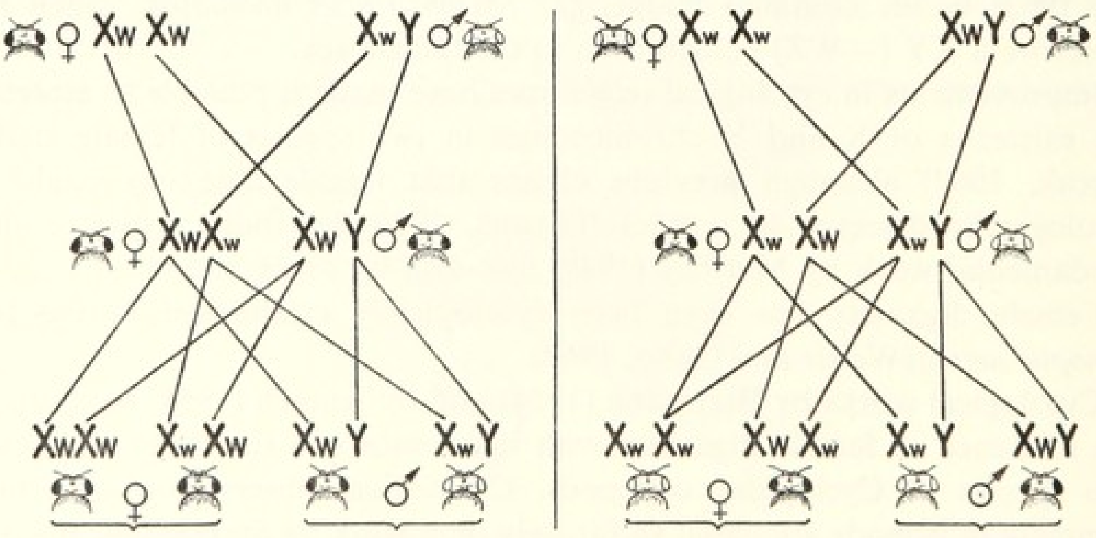

FIG. 4.5. Sex-linked inheritance in *Drosophila melanogaster*, a species showing male digamety.

half white-eyed and half red-eyed individuals both of the male and the female sex.

These results, which contrasted with those of all previous mendelian experiments, showed a criss-cross type of inheritance with the male that transmits the white eye colour to his grandsons through his daughters. The assumption that the gene for white eyes is located in the X chromosome and that Y carries no normal allele for white provided the explanation of this peculiar type of inheritance as shown in the attached diagram (Fig. 4.5). Xw indicates the presence of the normal allele in the X chromosome and Xw indicates the presence of its recessive allele for white eyes. The different phenotypes are indicated by white- and red-eyed *Drosophila* heads.

It has been shown that this type of inheritance is shared in *Drosophila melanogaster* by about 200 other genes which resulted later to be located in the X chromosome. Other instances of sex-linked genes have been described in insects showing male digamety: *w* (white) in *Culex molestus* (Gilchrist and Haldane, 1947), *r*, which is a partially sex-linked gene in *Chironomus pallidivittatus* (Beerman, 1955).

Colour blindness and most cases of haemophilia are X linked in man and quite a few sex-linked traits have recently been discovered in other mammals and especially in mice: Tabby (Falconer, 1952), scurfy (Russell *et al.*, 1959) and mottled (Fraser *et al.*, 1953). Yellow in cats (Bamber, 1927) and haemophilia in dogs (Hutt, Rickard and Field, 1948 and Mustard *et al.*, 1960) are also well-known examples.

Numerous examples of sex-linked genes have also been found in fishes (see Chapter 6) and an instance of sex-linked trait in plants has been discovered in *Melandrium* where it is connected with male digamety (Winge, 1931).

2. *Sex-Linked Genes in* Abraxas (*Female Digamety*)

The sex-linked inheritance in cases of female digamety was first studied by Doncaster and Raynor (1906) in *Abraxas grossulariata* in crosses of a recessive colour mutant *lacticolor* (g) with its normal allele *grossulariata* (G). The results were interpreted (Bateson and Punnett, 1911) with the assumption that *grossulariata* and its *lacticolor* allele are located on the chromosome X (Z chromosome of most authors) and that they are absent in the Y, or W, chromosome. The results of the crosses, that are similar to those obtained in *Drosophila*, are expressed in the diagram of Fig. 4.6 with the different phenotypes and genotypes.

Similar conditions are found in crosses of barred and non-barred individuals in poultry and a totally sex-linked gene *albus fuscus* has been discovered in the Isopod *Idothea baltica* by Tinturier Hamelin (1960).

American races of the brine shrimp *Artemia salina* also show female digamety, as demonstrated by the study of white eye, a mutant allele located in the X chromosome (Thompson Bowen, 1963).

3. One Sided Sex-Linked Inheritance

This type of sex-linked inheritance has been discovered in the fish *Lebistes reticulatus* (Winge, 1922). The gene *Maculatus* (Ma), that produces a black spot on the dorsal fin and a large red spot below the dorsal fin, is confined to the Y chromosome. Since no alleles of this gene exist in the X chromosome the Ma gene is transmitted from male to male only.

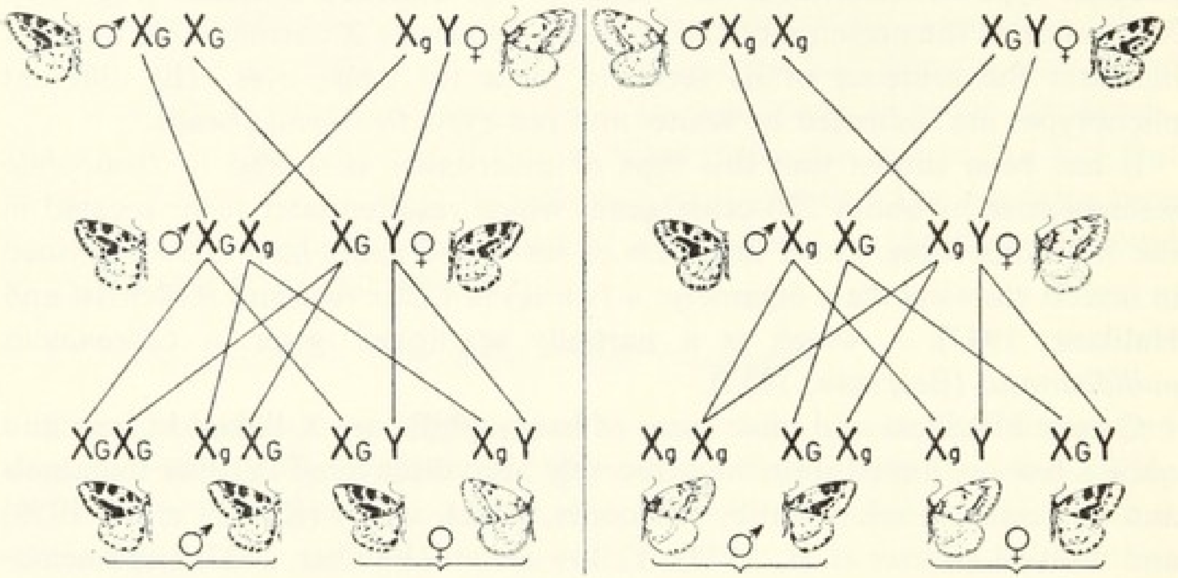

FIG. 4.6. Sex-linked inheritance in *Abraxas grossulariata*, a species showing female digamety.

The actual existence of completely Y linked genes in man has been the matter of some controversy. The often-cited pedigrees of porcupine men and of the webbed toes character should not be regarded, according to Stern (1960), as sure examples of completely Y linked inheritance. Recent evidence points on the other hand to complete Y linkage for some examples of hypertrichosis of the ear (Dronamraju, 1960, 1964) although autosomal inheritance has also been suggested on the basis of some researches.

4. Non-disjunction of the X Chromosomes in Drosophila

The peculiar way of transmission of the sex-linked traits supported thus the theory of sex digamety because the sex-linked genes are inherited as though they were located in the sex chromosomes. The conclusive proof of their location in the sex chromosomes was obtained through the study of non-disjunction of the X chromosomes.

Bridges demonstrated with his work on the effects on non-disjunction of the X chromosomes, which was concluded in 1916, that anomalies in the inheritance of sex-linked characters correspond to irregularities in the transmission of sex chromosomes and at the same time laid the foundation for the balance theory of sex determination.

The crosses of white-eyed females with red-eyed male *Drosophila* produced exceptional red-eyed male and white-eyed female individuals instead of the red-eyed females and white-eyed males. These exceptions were explained when it was demonstrated that during meiosis two X chromosomes may not be disjoined but exceptionally both remain in the egg or migrate to the polar body. In consequence eggs with two X chromosomes and eggs without any sex chromosome are produced.

The fertilization of these two sorts of eggs with X or Y bearing sperms give rise to four types of zygotes:

(a) zygotes with three X chromosomes (two with the white and one with the normal allele);

(b) zygotes with two X (both bearing the allele for white) and one Y chromosome;

(c) zygotes with one X chromosome (with the normal allele for red and without Y chromosome);

(d) zygotes without X chromosome and with the Y chromosome only.

The type (a) usually dies and (d) always dies but the (b) and (c) zygotes develop the exceptional white-eyed females and red-eyed males. This interpretation was also cytologically controlled in the abnormal individuals.

The white-eyed XXY females were crossed to normal red-eyed males and secondary non-disjunction was observed in 4 per cent of the progeny where a small number of daughters have white eyes and a corresponding percentage of males has red eyes.

The white-eyed females proved to have a XXY constitution with the 2X from the mother and the Y from the father and red-eyed males have a normal XY constitution because they have inherited the X chromosome with the normal allele for white from the male parent and Y from the mother. The other sex combinations include normal white-eyed males and normal red-eyed females, females with an additional Y chromosome, females with three X chromosomes (the so-called super-females), white-eyed males with two Y chromosomes and zygotes with two Y and no X chromosomes which are not vital.

Animals with Reversed Sex Phenotype and Subdioecious Plants

A few exceptional individuals of unisexual animal species may become hermaphrodite or may show a sex phenotype which is the reverse of their genotypic sex. Specimens of a male digametic species may thus develop a male phenotype under the action of environmental factors although they have an XX chromosomal constitution.

A similar phenomenon is recorded in subdioecious plants where a few hermaphrodite flowers and even a few flowers of the opposite sex may occur in genetically male or female plants.

Crosses obtained employing animals with reversed sex phenotype or subdioecious plants gave quite similar results and helped in establishing the type of sex digamety when neither heteromorphic sex chromosomes nor sex-linked traits could be detected.

1. Animals with Reversed Sex Phenotype

Witschi (1923b) found a few *Rana temporaria* hermaphrodites which showed functional testes and ovaries and obtained an all-female progeny of forty-five individuals through successful fertilization between sperms and eggs from hermaphrodite individuals. Eggs from normal female individuals were inseminated by hermaphrodite frogs and an offspring of 182 females was obtained. Eggs laid by hermaphrodite frogs were also inseminated with normal sperms and they produced 135 male and 132 female individuals in a perfect 1 : 1 ratio. The above results demonstrated therefore the digamety of the male sex in *Rana temporaria* and showed also that the hermaphrodite frogs employed in the crosses were genetically homozygotic females with partially reversed sex phenotypes. Crew had previously obtained (1921) only females (474 individuals altogether) in the progeny of a pair of frogs and the examination of the male partner had shown that it contained the remains of an ovary and that it was a genetic female with male gonads.

Crew himself made a similar but incomplete observation in fowls. An old hen, which had been a good layer, began at 3½ yr to assume male characters and a year later was able to fertilize a normal hen with sperms formed in its right gonad. Two chicks were produced, one of them a male, the other a female, just enough to confirm the digamety of the female sex (which on the other hand is demonstrated by experiments on sex-linked genes) but not enough to test the expected ratio of 2 ♀♀ : 1 ♂.

This result was obtained in the most convincing way in the Humphrey's experiments on the axolotl (1945, 1948). The presumptive territory of an embryonal male gonad was grafted on a female embryo at the place of its future left ovary. The graft differentiated into a testis and masculinized the female guest even in the right gonad and the ovary degenerated and formed testicular nodules. Normal phenotypic males were thus obtained and they were able to fertilize normal females. The offspring included female and male individuals in the approximate proportion of three female to one male individual. This result demonstrated the female digamety of the axolotl.

Two-thirds of the females obtained from this cross must carry XY (or WZ) chromosomes, and one-third YY (or WW) sex chromosomes. To check this conclusion seventeen females of this generation were selected at random and crossed with normal males (that carry XX or ZZ chromosomes). Six of them gave a completely female progeny according to the expectation:

$$\text{YY } (\female) \times \text{XX } (\male) = 100 \text{ per cent XY } \female \text{ (833).}$$

Eleven of them gave half male and half female progeny, that is:

XY (♀) × XX (♂) = 50 per cent XY ♀ (378) + 50 per cent XX ♂ (370). Eleven to six fits quite well with the expected ratio of 2 : 1.

Masculinization of YY females with the grafting method made possible to cross such neomales with normal females and exclusively female offspring was thus obtained according to the expectation:

YY (neo ♂) × XY (♀) = XY (♀) + YY (♀).

Similar experiments were carried on by Gallien (1954) on *Pleurodeles waltlii*, a newt, and on a lower Anuran, *Xenopus laevis* by Gallien (1956) and by Chang and Witschi (1960) following administration of estradiol (a female hormone) to larvae of both species which changed genetic males into females.

Six neofemales of *Pleurodeles* crossed to normal males gave birth to 940 male individuals and the same results were obtained in *Xenopus*. Witschi (1960) stated in a recent report that four generations of purely XX individuals have been obtained in *Xenopus* through treatment of some of them with extradiol in order to secure the presence of a few functional females with XX chromosomes (that is with the sex chromosomes of the male sex) for the reproductive processes. A detailed cytological analysis in *Xenopus* eventually demonstrated the existence of heteromorphic sex chromosomes (X and Y) in normal female (Weiler and Ohno, 1962).

It must be pointed out that the existence of morphologically detectable sex chromosomes in many amphibian species has been the subject of some controversy (Galgano, 1933; Matthey, 1949; Wickbom, 1945; Witschi, 1924, 1956) and it must be concluded that well differentiated sex chromosomes are rare among amphibians. Some of them show male and some others female digamety, a situation that points to a slight differentiation between the X and the Y chromosome.

Winge (1930) obtained in the course of his experiments on the cyprinodont fish *Lebistes reticulatus* some XX male individuals whose chromosome constitution was clearly demonstrated by two pairs of marker genes (Fig. 6.1). Such functional males—when crossed with normal females—produced only female individuals and thus the female homogamety of the normal strains of *Lebistes* was demonstrated. The male digamety of the fish *Oryzias latipes* was established by Aida (1921, 1936) and Yamamoto (1953, 1955, 1960) obtained, by treatment with estrone and stilbestrol, sex reversal into functional females of individuals with a male chromosome constitution (XY) which was marked —as in the above-mentioned example of *Lebistes*—by genes of male type coloration. The results confirmed the previous conclusions. Males of *Oryzias* with a YY constitution were also obtained, thus confirming previous results in *Lebistes*.

Recent research on the isopod *Idothea baltica* (Tinturier Hamelin, 1960) has shown the existence of males having an exceptional XY constitution which is indicated by the presence of the marker gene giving the phenotype *albus fuscus* which is localized on the Y chromosome. Crosses indicate a normal female digamety with the formation of a few exceptional reverted sex phenotypes which are indicated by the marker genes as in the classic examples of the cyprinodont fishes. I am informed by Bocquet and Tinturier Hamelin that crosses between nineteen exceptional females with XX chromosomes and normal males has produced a progeny of 2104♂♂ and 277 ♀♀. The result thus substantially confirms the following genetic equation:

$$♀\,XX \times ♂\,XX = ♂♂\,XX.$$

Winge (1931) and later Yamamoto (1959) pointed out that in cyprinodont fishes intersexes are rare although sex-reversed phenotypes are easily obtained in laboratory. The same seem to occur among Isopods. This is an important point for the discussion of monogeny and altered sex ratios among fishes and Isopods.

2. *Sex Genetics of Subdioecious Plants*

Kuhn's and Gabe's independent works (1939) made decisive contributions to our understanding of sex genetics of subdioecious plants.

Kuhn selfed subandroecious plants of *Thalictrum fendleri* and they produced seventy-two male and twenty-two female plants (1 ♀ : 3 ♂♂). The males were shown to belong to two different types when crossed to normal females because two-thirds of them (36) gave male and female progeny in equal proportion and one-third (16) gave only male progeny having a YY constitution. Selfed subgynoecious plants of *Thalictrum dasycarpum* and *Thalictrum polygamum* gave female plants only. These results are easily explained by assuming that both male and subandroecious plants have the constitution XY.

Similar conclusions were reached by Gabe (1939) with crosses of *Mercurialis annua* and by Rick and Hanna (1943) with *Asparagus officinale*. None of the species used in experiments of this kind show any heteromorphic sex chromosomes and sex digamety is thus established only on genetical ground. Subdioecious plants can thus be considered the phenotypic equivalents of the previously mentioned animals where phenotypic sex reversal is easily obtained either in nature or by artificial methods.

Earlier statements, reported by Correns (1928), that selfed subandroecious plants give male progeny and on the contrary subgynoecious plants give female progeny, when selfed or intercrossed are considered as based on insufficient data (Westergaard, 1958).

Gynandromorphism

Normal male or female type structures, which form cellular districts that have different chromosomal constitution and show a sharp line of demarcation, may be present in the same individual. Sometimes the left half of the body is female and the right half male (Fig. 4.7), sometimes just a quarter of the body is male and the rest is female or just a small patch appears to be male in a female body. Such individuals, where no intermediate conditions but only pure male or female territories can be observed, are known as gynanders or gynandromorphs.

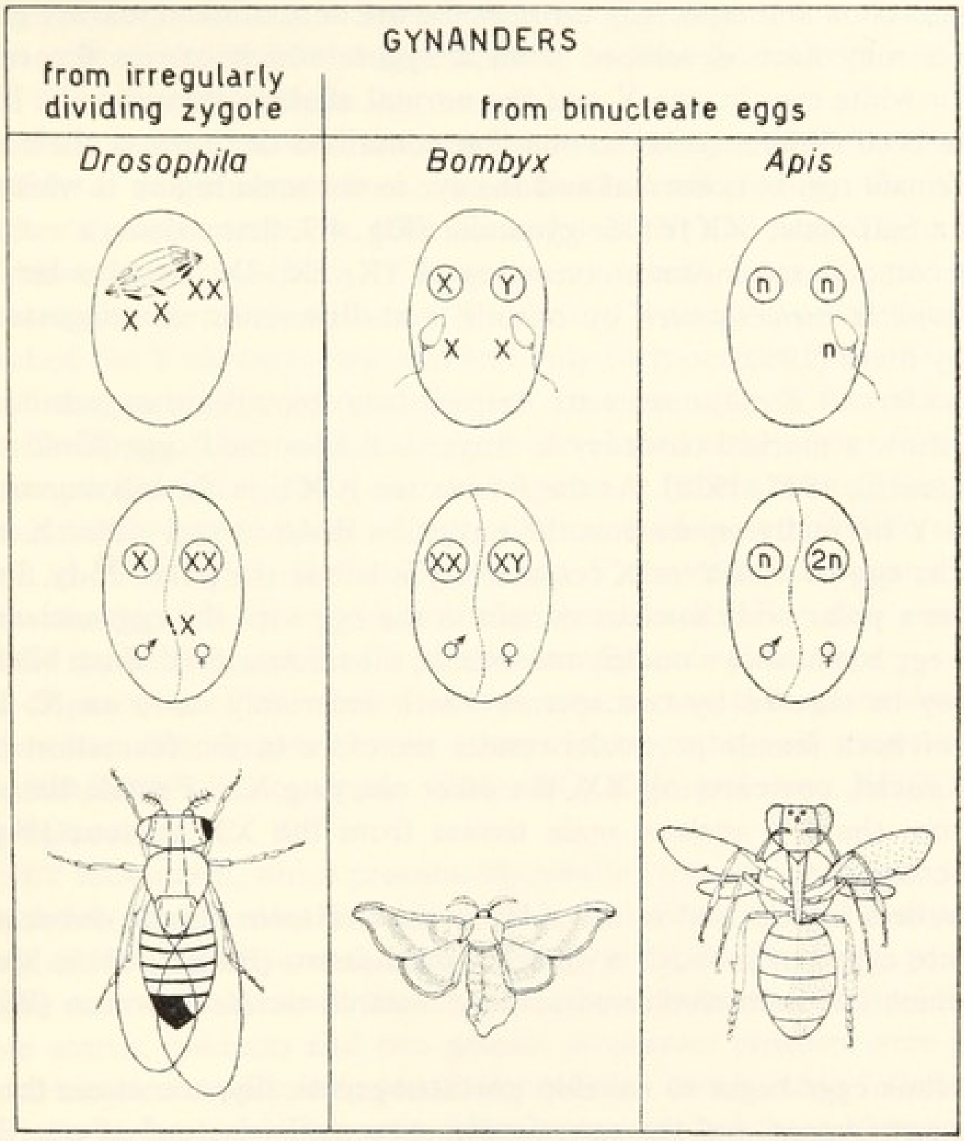

Fig. 4.7. A diagram illustrating the origin of gynandromorphism in three different groups (based on various authorities).

Gynandromorphism has especially been studied in *Drosophila* (Morgan and Bridges, 1919), in *Bombyx* (Goldschmidt and Katsuchi, 1927) and in bees (Mehling, 1915; Rothenbuhler, 1957) and it has been one of the most convincing pieces of evidence in the early days of the chromosome theory of sex determination. *Drosophila* gynanders are originated by elimination of an X chromosome. A gynander begins its development with two X's but in the course of cleavage an X is eliminated by one of the blastomeres. If the irregular chromosome division takes place during the first cleavage division half of the animal will develop from the blastomere with two X's and will be female, the other half with XO constitution will become male. If the elimination occurs later the blastomere that contains one X only will give rise to less than half the body and the later the elimination the greater the differences in size between the male and female parts.

The proof of the X chromosome elimination is obtained through cytological examination and especially through the use of sex-linked marker genes. A gynander may have developed from a zygote which carries the recessive allele for white eyes in one X and the normal allele in the other X. If the X that has been eliminated is the one that contained the normal allele the eye in the female region is normal and the eye in the male region is white in the case of a half male, half female gynander (Fig. 4.7, first column).

Very complicated mosaics consisting of 1X, 2X, 3X can also be formed in *Drosophila melanogaster* by mitotic non-disjunction as suggested in a paper by Stern (1960).

Gynanders in *Bombyx mori* are derived from binucleate eggs and certain strains show a marked tendency to form such abnormal eggs (Goldschmidt and Katsuchi, 1927, 1929). As the female sex is XY in the silkworms the X and the Y normally separate at the reduction division and either X or Y is left in the egg so that Y or X respectively is left in the polar body. In some instances a polar body nucleus remain in the egg with the egg nucleus itself and the egg has now two nuclei, one with X, the other with Y. Such binucleate eggs may be entered by two sperms which invariably carry an X. Fertilization of both female pronuclei results therefore in the formation of two diploid nuclei, one carrying XX, the other carrying XY. Female tissues will arise from the XY nucleus, male tissues from the XX nucleus (Fig. 4.7, central column).

Gynanders in bees and in other Hymenoptera seem also to develop from binucleate eggs but through a different mechanism (Boveri, 1915; Mehling, 1915) which has been confirmed also by research on *Habrobracon* (Whiting, 1928a).

Sometime eggs begin to develop parthenogenetically, sometime the polar body is not formed and the eggs contain two nuclei instead of one. When, as usual, a single sperm enters the binucleate egg one nucleus only is fertilized and the other remains haploid. As haploid eggs of Hymenoptera develop into males (Chapter 10) and diploid eggs normally develop into females, the unfertilized nucleus gives rise to male tissues, the fertilized nucleus to female tissues (Fig. 4.7, third column).

The various examples of gynandromorphism among insects demonstrate therefore the strict adherence of the sexuality of extended body regions to the chromosomal constitution with the formation of mosaics where the action of sex differentiating substances appears not to be diffused beyond the boundary of each cellular element.

The production of diffusible sex hormones (Chapter 9) does not allow the formation of clear-cut sexual mosaics in man and in other vertebrates. A number of well-investigated sex mosaics is, however, being described in the literature concerning sex chromosomes in man.

Attempts to classify the various types of human mosaics seem to be premature at the present stage of investigations. Some of them have originated through non-disjunction and some others through loss of sex chromosomes in early or later stages of the development so that generalized and partial mosaics have been described.

One of the earliest known instances has been interpreted in terms of non-disjunction of sex chromosomes. An individual affected by the so-called Klinefelter's syndrome (see p. 109) showed a XXY/XX mosaic (Ford *et al.*, 1959). More than two-thirds of bone marrow cells from the patient had forty-seven chromosomes with two X and one Y and the other third of the cells lacked the Y chromosome and had only forty-six chromosomes, that is they had the normal female complement. It appears likely that this mosaic patient developed from an XXY zygote developed either from fertilization of an exceptional XX egg by a normal Y sperm or from a normal X egg by an exceptional XY sperm. Most of the cells involved in sexual differentiation were left as XXY before the initial loss of a Y chromosome occurred giving thus rise to the line of chromosomally female cells. For this reason the XXY/XX mosaic has assumed a predominantly male phenotype.

Non-disjunction of sex chromosomes, which may take place during the first divisions of the zygote or may occur in later embryonic stages, can give rise to XO/XXX (Jacobs *et al.*, 1960), XO/XXY and even XO/XX/XXX mosaics (Jacobs *et al.*, 1961).

XO/XY mosaicism, which presumably results from the loss of one chromosome was first described by Hirschhorn and collaborators (1960) in a baby 3½ yr old that showed an apparently male phenotype. XO cells in the proportion of 60 per cent were obtained from bone marrow cultures. Vagina and penis but also uterus, oviducts and two gonads in ovarian position were present. Gonadal biopsy showed unripe testicular tubules, two ovarian follicles and a stroma of ovarian type. The patient has been regarded as an hermaphrodite although there was no indication for a future normal development of both male and female sex cells in the gonads.

Numerous examples of XO/XY gynandromorphism have been described since (Bompiani *et al.*, 1963) and their analysis will help much in understanding the inner mechanisms of sex determination in man.

Environmental and Genetical Influences on the Migration of the Sex Chromosomes

1. The Effect of Temperature on X in Talaeporia

The work of Seiler on the moth *Talaeporia tubulosa* (1920) has given one of the most impressive demonstrations of the indirect influence of external factors on sex determination through their action on the migration of sex chromosomes (Fig. 4.8). Fifty-eight autosomes and a single X chromosome are present in *Talaeporia* females. During the egg maturation the orientation of the chromosomes on the spindle is such that the X chromosome tends to remain in the egg when the females are maintained at a temperature above 30°C. When the temperature is below normal (3–5°C) the single sex chromosome shows a marked tendency to pass into the polar body. Since the eggs are the sex-deciding gametes the high temperature induces the formation of about 61 per cent males, the low temperature favours instead the production of about 61 per cent females. Over-ripeness of eggs favours also the permanence of the X chromosome in the eggs and thus produces a majority of homozygous male zygotes.

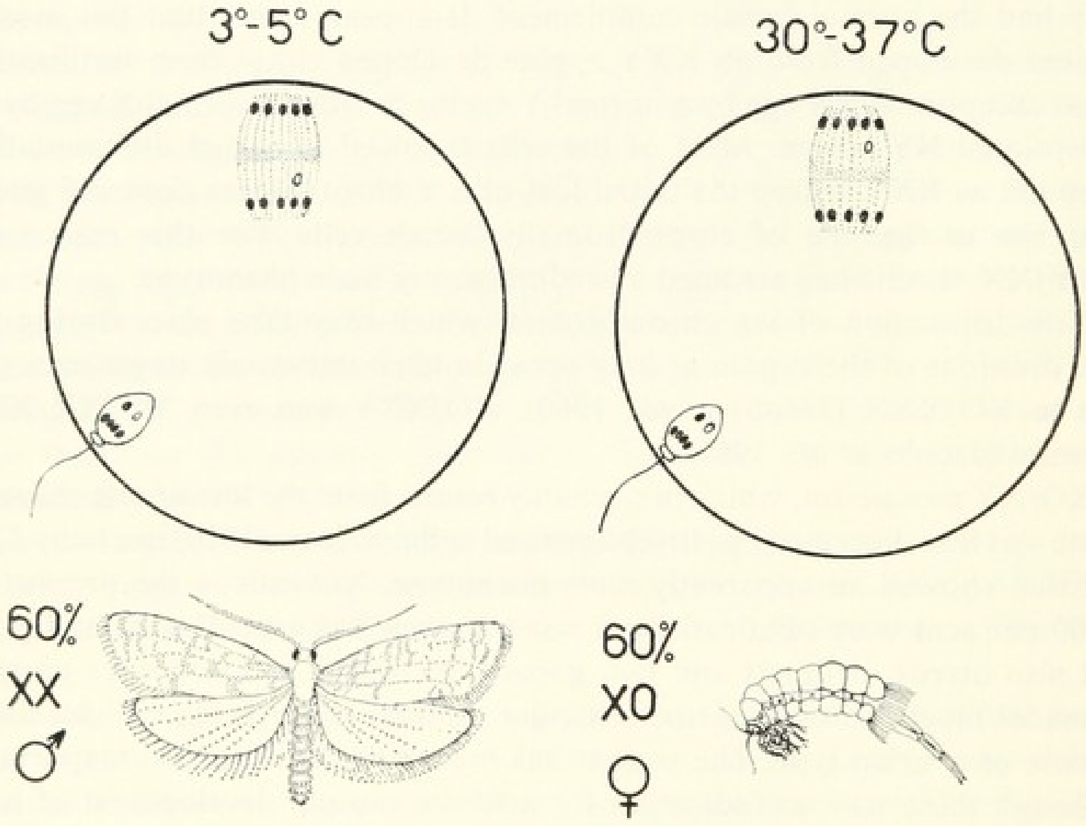

FIG. 4.8. Migration of the X chromosome and sex determination in the moth *Talaeporia tubulosa*: the single X chromosome of the oocyte has a tendency to migrate into the polar body when the females are kept at a high temperature. A high percentage of females is thus obtained (Seiler, 1920).

Thus environmental factors may alter the sex ratio by influencing the segregation of the X chromosome and, as in the previous instances, it is shown that the abnormal behaviour of the sex chromosome is clearly expressed in the abnormal sex ratios.

Researches by Metzler (1957) who investigated the external influences on sex ratio in the copepod *Cyclops viridis* have yielded results that resemble very much those obtained by Seiler in *Talaeporia. Cyclops viridis* shows female digamety and the number of males increases with the increase of temperature: at 2°C 112 male and 179 female individuals are obtained, 201 males and 138 females are obtained from cultures at 23°C. Hunger, maintenance of the mothers in culture water at pH 6, their treatment with ultraviolet rays also produce a significant increase in number of males. As the action of the above factors is particularly evident a few hours before egg laying, the author concludes for what in Hartmann's nomenclature is called a case of genotypic sex determination.

Although only the cytological control could give the decisive proof in favour of external factors influencing the behaviour of the sex chromosome, Metzler's interpretation appears quite convincing at the present stage of the research.

The Seiler experiments appear also to have a considerable significance in interpreting the abnormal behaviour of sex chromosome maturation divisions during the life cycles of various heterogonic species.

2. *Sex Determination Through Chromosome Elimination in* Sciara

The most striking peculiarities in the behaviour of the sex chromosomes have been observed among lower Diptera, especially among Cecydomyidae or gall midges (White, 1947) and among Sciaridae or fungus gnats.

The Sciaridae have been accurately investigated by Metz (1938) and coworkers in a series of researches which represent one of the finest examples of integration of genetic with cytological work in the study of sex determination.

Sciara coprophila is the best-known representative of the genus whose species behave in much the same way both during gametogenesis and during early development.

The zygote of both male and female individuals contains three pairs of autosomes, three X chromosomes and three elements which are called limited chromosomes because they are found only in the germ line and not in the soma. Such elements are apparently empty as regard genetic material but they undergo all the essential mitotic and interphase transformations.

The original chromosome set is maintained through only five or six cleavage divisions after which the limited chromosomes are eliminated from the nuclei having somatic destination.

Sexual differentiation takes place soon because two paternal X chromosomes are eliminated from the somatic line during mitosis in the embryos that are destined to become males. Only one paternal X chromosome is eliminated from the somatic set in the corresponding mitotic division of female individuals.

Thus male and female somatic cells become differentiated according to the formulae XX = ♀ and XO = ♂ but the cells of the germ cells retain three X chromosomes both in the male and in the female individuals.

A third elimination takes place in each individual after the germ cells have reached their site in the gonads when one paternal extra chromosome passes through the nuclear membrane into the cytoplasm of both spermatogonial and oogonial cells.

The sex chromosome complement of the male and female germ cells remains thus apparently identical after this elimination as they possess two X chromosomes one of which is of paternal and one of maternal origin.

Oogenesis in *Sciara* is quite normal with random segregation of the chromosomes that have undergone regular synapsis and crossing-over. The chromosome complement of the ripe egg consists therefore of three autosomes, one X and one or more limited chromosomes.

Spermatogenesis is quite abnormal and results in the differential segregation of the maternal autosomes and X chromosome from the homologous elements of paternal origin. A unipolar spindle is formed at the first spermatocyte division with all the paternal chromosomes degenerating. All the limited chromosomes, however, migrate to the single secondary spermatocyte with the chromosomes of maternal origin. The second maturation division is normal for all chromosomes except for one X chromosome (Crouse, 1943) which goes undivided to one pole before anaphase has commenced for the remaining elements. After the division has taken place one set contains the X chromosome and becomes the nucleus of a functional sperm and the other set degenerates.

As a result of the different meiotic processes during gametogenesis the egg provides to the zygote a single X chromosome and the sperm brings two X chromosomes which derive from the X chromosome that was left undivided during the second meiotic metaphase. No visible differences have been detected between zygotes forming male and female individuals and therefore the inner mechanism of sex determination must be due in *Sciara* to some morphologically undetectable genetic peculiarity.

Two X chromosomes are eliminated from the somatic line of male individuals and only one X from the somatic line of females and all subsequent differences in spermatogenesis and oogenesis seem to be the consequence of such initial different chromosome behaviour. Temperature differences are responsible for the different behaviour of the X chromosome in *Talaeporia* and it appeared reasonable to assume that the elimination of one or two X chromosomes in the female and in the male genotypes is due to some genetic factor which is actually responsible for sex determination in *Sciara*: thus what are regarded as errors in mitosis in most species have become normal mechanisms of sexual determination in *Sciara*.

Metz showed that some fertilized females give only female and others only male progeny. The progeny of most females includes, however, both sexes in a ratio which varies through a wide range. Metz distinguished therefore unisexual and bisexual families and assumed that female producing females (they may be called thelytokous females) are heterozygous for a mutation, which is localized in one of the X chromosomes and is responsible for a modification of the cytoplasm of the first blastomeres causing the expulsion of a single X. Males and male producing females (they may be called arrhenotokous females) are on the contrary homozygous for the normal allele whose presence causes the expulsion of two X chromosomes.

The analogy between the external influences that in some species induce chromosome elimination and the genetic mechanism suggested by Metz are striking and it looks as if the action of environmental factors had in some way been integrated and canalized in a precise sex determining system (see Chapter 12).

Crouse (1960) has confirmed Metz's conclusion and has proved that elimination at the critical stages in the embryo is actually controlled by the heterochromatin that normally lies adjacent to the X centromere.

The cytological and genetical work carried on in *Sciara* has thus provided on one hand a most convincing example of the influence of the sex chromosomes on sex differentiation and provides on the other hand a model for the interpretation of abnormal chromosomal behaviour in heterogonic species, where the sequence of sex phenotypes is often based on similar mechanisms.

CHAPTER 5

INTERSEXUALITY AND THE BALANCE THEORY OF SEX

Intersexes and Supersexes in *Drosophila melanogaster*

THE work by Bridges on triploid intersexes in *Drosophila melanogaster* (1921–1939) provided the clearest example of a quantitative relation existing between male and female sex determiners located in sex chromosomes and in autosomes. For this reason the sex determining mechanisms in *Drosophila* (Fig. 5.1) are discussed at the beginning of this chapter although the concept of intersexuality was first established by Goldschmidt (1915) in the course of his *Lymantria* work and a first idea of a dosage effect between in male and female factors was also advanced by Goldschmidt in 1912.

1. The Sexual Function of Y

The Y chromosome of *Drosophila melanogaster* is slightly smaller than X and in other species of the genus all gradations in the relative size of the Y are found between a condition of equal length and the condition where Y is totally absent. The size difference between the two chromosomes does not, however, give any evidence regarding the function of X and Y in the sex determination of *Drosophila*.

Morgan's researches on sex-linked inheritance showed that the Y behaves in *Drosophila melanogaster* as though it were practically empty of genes. The few genes discovered so far in the Y are situated near the centromere in the restricted region where crossing-over with X is still possible.

The experiments of Bridges (1916–1921) on the disjunction of the X chromosomes demonstrated that individuals of *Drosophila melanogaster* with XXY are normal females and that individuals with a single X and no Y show a normal male aspect.

The Y chromosome is thus lacking in male determining factors: it possesses a well localized gene, or a gene complex, which influences the male fertility by acting on the motility of spermatozoa (Stern, 1929) and Stern and Hadorn (1938) demonstrated through transplantation experiments that sterility is not due to the genetic constitution of the gametes.

2. *The Progeny of Triploid Females*

Triploid females ($3n = 3X + 3A$) seem to have originated from exceptional diploid eggs that were fertilized by normal haploid sperms. They can be distinguished from the normal diploid females especially for their heavy thick-set bodies, coarse bristles and coarse facetted eyes. They show otherwise normal female characters and appear to be normally fertile.

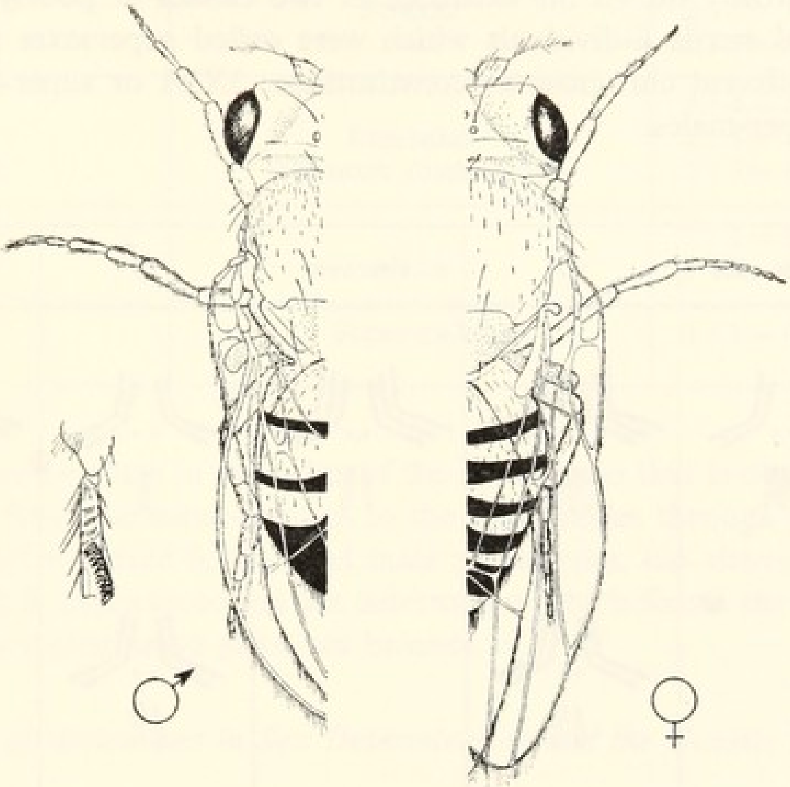

FIG. 5.1. Male and female *Drosophila melanogaster*, note the differences in the abdominal segments and the presence of sex combs in the fore legs of the male.

A female of this type produced, in the course of a research by Bridges (1921), nine normal male, ninety-six female individuals and thirty-seven exceptionally large individuals which showed various body malformations (in the eyes, in the bristles, in the wings) and proved to be totally unfertile. They were especially remarkable for their poorly developed gonads and ducts. The external genitalia often appeared a mixture of rudimentary male and female parts and in some of them one ovary and one testis were present together. Sex combs presented various degrees of malformation, colour and shape of the abdomen appeared as a patchwork of male black and female pale colour. In this series of intersexes, which showed an extremely variable mixture of male and female characters, "nearly male" and "nearly female" specimens were present but they could always be distinguished from normal male and female individuals because of their resemblance to triploid individuals.

They could be divided into male type and female type intersexes and cytological and genetical analysis demonstrated that the former possessed three sets of the II and III chromosomes, two sets of the IV chromosomes and two X chromosomes only, the latter three sets of autosomes (chromosome IV included) and two X chromosomes. Presence or absence of Y exerted no influence in sex expression of either class of intersexes.

Previous experiments concerning the non-disjunction of the X chromosome had already shown the existence of two classes of poorly viable, late hatching and sterile individuals which were called supersexes and showed extremely different chromosome constitutions: 3X2A or super-females and 1X3A or super-males.

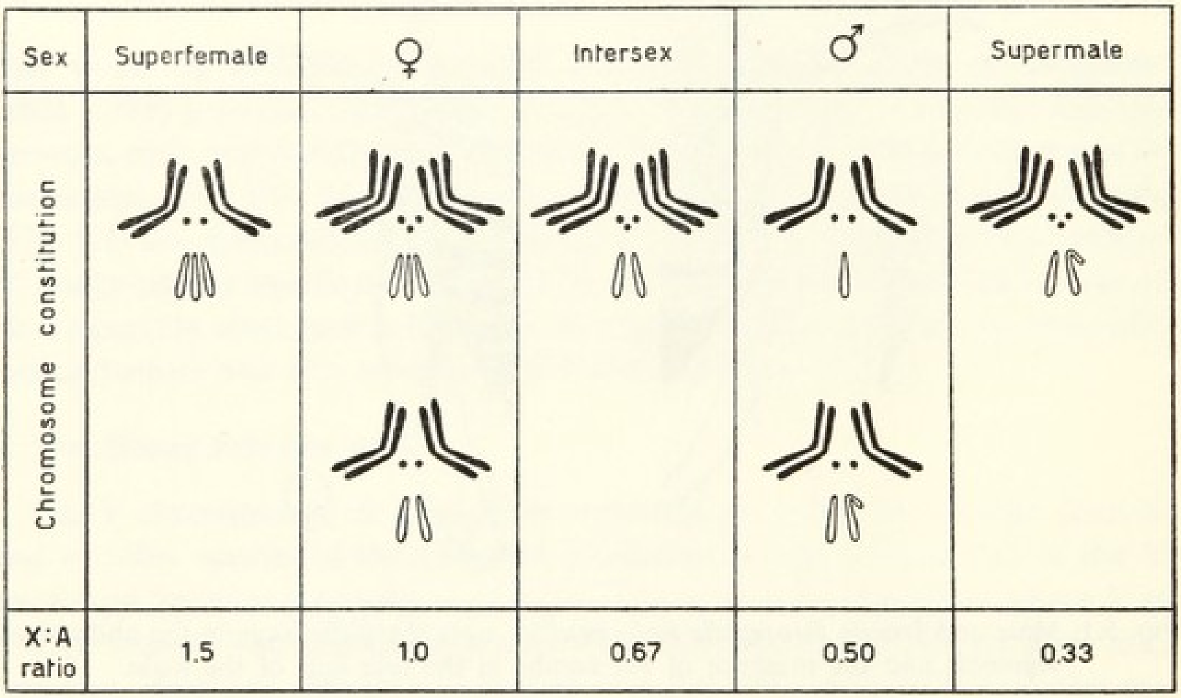

FIG. 5.2. Diagram showing the ratios between X chromosomes (outlined) and autosomes (solid) and the corresponding sex phenotypes in *Drosophila melanogaster* (after Bridges).

The sexual characters of these individuals are not strikingly more marked than those of their normal diploid brothers and sisters and therefore it has been suggested to call them ultra-males and ultra-females, rather than supermales and super-females. Table 1, which has also been summarized in Fig. 5.2, shows the various sex phenotypes, and their corresponding chromosome constitutions, as they were discovered by Bridges in the course of his investigations on sex determination in *Drosophila melanogaster*.

In Table 1 are also haploid females and triploid and tetraploid females with unaltered ratio between X chromosomes and autosomes which appear to be normal in sex characters.

TABLE 1. CHROMOSOMAL FORMULAE AND SEX PHENOTYPES IN *Drosophila melanogaster*

Chromosomal formulae	Sex phenotypes	X : A ratios
3X 2A	Super-females	3 : 2 = 1·5
4X 4A	Females	4 : 4 = 1
3X 3A		3 : 3 = 1
2X 2A		2 : 2 = 1
1X 1A		1 : 1 = 1
3X 4A	Intersexes	3 : 4 = 0·75
2X 3A		2 : 3 = 0·67
2X 3 (—IV)	Intersexes (male type)	2 : 3 = 0·67
1X 2A	Males	1 : 2 = 0·5
2X 4A		1 : 2 = 0·5
1X 3A	Super-males	1 : 3 = 0·33

The regular decrease in the value of the X : A ratio that is observed in the series going from the super-females to the super-males through the series of intersexes and standard female and male phenotypes, has shown a relationship between X and autosomal sex determiners which forms the basis of the theory of the quantitative genic sex balance.

3. The Role of Autosomes in Sex Determination and the Genetic Balance

The increasing proportion of autosomes in the various chromosome complements shown in Table 1 corresponds to an increase of male characters in the different sex phenotypes and these observations confirm the previous conclusion that autosomes, and not only X chromosomes, play an important role in the determination of sex.

This role is due in Bridges interpretation (1939) to an internal preponderance of "male tendency" genes in the autosomes of *Drosophila*. The net effect of genes in the X chromosomes results, on the contrary, in a tendency to the production of female characters.

It was demonstrated in this way that the early scheme which identifies the sex determining mechanism with a simple back-cross mechanism is purely formal and inadequate and sex is not simply determined by a couple of genes that are carried by the sex chromosomes.

As every male or female individual of *Drosophila* carries both autosomes and X chromosomes male and female potencies are present in its genotype and therefore the assumption of the AG complex (see p. 69) appears quite superfluous in the *Drosophila* sex determining mechanism. The quantitative relation, or the balance, between the male and female factors decides the sex phenotype of each individual.

The absence of a sex role of the Y chromosome makes the principle of sex balance particularly simple and clear in the case of *Drosophila melanogaster*.

The quantitative approach to the problem of sex determination in *Drosophila* confirmed also a principle that had been previously demonstrated by Goldschmidt. If the ratio between the number of X chromosomes and of sets of autosomes is intermediate between 1·0 and 0·5 the resulting individuals are neither normal males nor females, and therefore a given threshold must be reached if normal male or female phenotypes are to be obtained.

Objections have been made to the expression "genic balance" which ought to be called a "balance of genic actions" (Goldschmidt, 1955) as the decision on male or female differentiation in the individuals seems actually to depend on a balance of sex differentiators (or inductors) (Witschi, 1960).

The discovery of the inner mechanism of sex digamety which is based on the genetic bipotentiality of both sexes and the consequent theory of balance, provided thus the explanation of the intersexuality phenomenon which is interpreted as the result of a disturbed balance of the male and female factors in the same individual.

4. Sexual Bipotency and Sexual Formulae

Bridges' experiments on *Drosophila* intersexes allowed the formulation of some sexual formulas where the dualism between the AG complex and the sex realizators was eliminated.

The sex potency of the X chromosome can be indicated with F and the sex potency of an haploid set of autosomes is indicated with M. Now if it is assumed that $F < MM$ and $FF > MM$, an XAA or FMM individual is a male and an XXAA individual is a female.

Such formulae FFMM = ♀ and FMM = ♂ can have wider application if a certain value is given to an f which may be located in the Y chromosome. The f factor has a O value when, as in *Drosophila*, sex genes are absent, it has a weak positive value when, as in *Rana*, it has a weak female genic content, and it has a negative value when, as in some fish and mammals, it has a content of male determiners. Thus the following formulations have been proposed for diploid organisms showing male digamety: FfMM = ♂; FFMM = ♀.

In the organism with female digamety the situation is reversed with the following formulae: MMFF = ♂; MmFF = ♀, with $MM > FF > Mm$.

Such formulae implicitly contain the cytological formulae XY = ♂ (or WZ = ♀) and XX = ♀ (or ZZ = ♂) which may become XO = ♂ and XX = ♀.

Such formulations are extremely generalized and have therefore very little practical value although they well express the fact that each sex genotype contains the potentiality of the other sex.

It is nevertheless very important to know the real distribution of the male and female sex determining factors within the X chromosomes and within the autosomes of *Drosophila,* that is whether the X chromosome contains a single F locus and whether M determiners are distributed over all autosomes in *Drosophila.*

It is possible that in some species only single F and M loci exist. The mechanism discovered in *Drosophila,* is nevertheless very important in understanding most cases of sex determination.

5. The Search for Female Sex Factors in the X Chromosome

Dobzhansky and Schultz (1931, 1934) added or subtracted segments of the X chromosome to normal diploid males or females of *Drosophila* or to triploid intersexes in order to investigate its sexual content. The use of suitable gene markers allowed to evaluate the extent of each fragment of X.

Most of the experiments were carried on triploid intersexes because the vicinity of their sexual balance to the critical thresholds for maleness and femaleness makes them particularly sensitive to the action of both genetical and environmental factors (Dobzhansky, 1930). Duplications and deficiencies induced, however, detectable effects in diploid male and female individuals also.

It was established that the addition of a fragment of X to 2X3A individuals always shifts intersexuality in the female direction and that the extent of the shift is roughly proportional to the length of the broken fragment.

Individuals with deficient X showed on the contrary a shift of intersexuality in the male direction.

Long duplications conferred to diploid females the appearance of superfemales and the appearance of intersexes to diploid males.

Only the short pairing segment proved to be devoid of sexual influence in either direction.

Dobzhansky and Schultz concluded from their experiments that the X chromosome in *Drosophila* does not contain a single F locus but it contains multiple female sex determiners which are located throughout its length with the exception of the pairing segment. Even if male sex determiners are also present in the X chromosome, they are located in the vicinity of stronger female determining factors. Therefore the single sections of X are female determining as the whole X chromosome.

The Dobzhansky and Schultz experiments raised the problem whether the multiple factors influencing sexual differentiation must be considered as sex modifiers or as proper sex factors. In other words some perplexity arose in deciding whether the factors located in the fragments of X chromosomes simply modify phenotypic characters whose presence might actually be determined by another factor (Punnett, 1933). Goldschmidt (1935) put the question in the same way and suspected that regions of X not yet appropriately explored by Dobzhansky and Schultz might actually contain the F determiner and—at a time when the separation between pairing and differential segments was not yet well realized—objected that a mechanism of multiple sex loci appears a poor thing for so constant a thing as sex determination.

The identity of the multiple factors with the sex factors acting on the sex balance mechanism was nevertheless maintained on the ground that the alleged modifying factors show the same quantitative relations as shown by the sex factors proper in the experiments by Bridges and that the segments of X show distinct additive action (Dobzhansky, 1935).

Pipkin (1942) studied other duplication intersexes possessing 2X3A and substantially confirmed the previous experiments as the addictions of male segments produced small or no shifts in the female direction, and longer sections produced marked shifts in the same direction. It was remarked on the other hand that the part to the left of section 17 has more feminizing influence than the part to the right.

On the basis of this result, however, Goldschmidt (1955) still distinguished a modifying action of the small fragments from the primary sex determination of sufficiently large segments of the X chromosome. The distinction is derived on one hand from Goldschmidt's peculiar conception that assumes the existence of a hierarchical organization of the genetic material and on the other hand it is partially based on the idea that the heredity of sex is completely different from all others.

Pipkin's experiments disproved nevertheless the possibility of the existence of a single female locus in *Drosophila*, a possibility that exists nevertheless in the sex determining mechanisms of other organisms.

The problem of the distinction to be made between sex genes and sex modifying genes is still open for some cases.

The term sex gene is replaced in most of the works on sex determination by terms such as sex modifier, sex determiner, sex factor or sex realizator with different implications which often disclose the authors' reluctance to regard the sex gene or the sex genes as ordinary genes. Goldschmidt (1955) clearly expressed this view by writing that the heredity of sex is completely different from all others.

It is certain that some factors that have been discovered in the search for male genes in the autosomes of *Drosophila* are modifying genes and not sex genes. The possibility of the assimilation of sex modifiers to sex genes and consequently the connection between the heredity of sex and the heredity of other characters will be discussed later (see Chapter 12).

6. *Autosomal Sex Genes and Modifiers*

A search for sex genes in the second chromosome of *Drosophila melanogaster* was carried on by Pipkin (1947) using the translocation and triploid method but it gave negative results. Also 3X3A females carrying a long section of the third chromosome showed no shift toward intersexuality and 2X3A intersexes plus long regions of the same chromosome were usually of extreme male type intersex but the shift to maleness appeared to be not significant (Pipkin, 1960). Pipkin deduced therefore from her experiments that intersexuality in 2X3A triploid intersexes depends on a dosage change involving both the second and third chromosome. A detectable determining influence of the single chromosome IV had already been ruled out by the work of Bridges.

It appears therefore that even if multiple sex genes are present in the autosomes as they are in the X chromosomes they are nevertheless so much diluted among the three autosomes that their influence cannot be demonstrated with certainty even in long regions of the 2 and the 3 chromosomes if strict criteria of evaluation are used.

It is nevertheless significant that a number of mutant loci which can produce different types of intersexuality, abnormal genitalia and sterility in female individuals, and are therefore male determining, have been detected both in the second and in the third chromosome. Goldschmidt regards such genes as sex modifiers rather than as proper sex genes, as some geneticists do.

Sturtevant (1920, 1921) isolated in the second chromosome of *Drosophila simulans* a recessive gene that converts diploid females to male-like intersexuals which show rudimentary gonads, ovopositor of abnormal form and two spermathecae.

A recessive transformer gene (*tra*), that totally changes diploid females into sterile males was also discovered by Sturtevant (1945) in the third chromosome of *Drosophila melanogaster*. Gowen and Fung (1957) have shown later that the transformer gene belongs to the same allelic series of Hr (hermaphroditism), a dominant gene which also affects diploid females (Gowen, 1942). They are transformed into individuals with sterile gonads of predominantly ovarian type which show complete male secondary characters with their female counterparts. As some parts of the gonads showed an appearance of testicular structure Gowen considered the *Hr* females as hermaphrodites although they cannot be considered as such according to the definition accepted by most authors (see p. 22).

Peculiar sex phenotypes have been detected in the course of the experiments made by Gowen and Fung in order to study the effect of *Hr* in combination with its normal allele and the *tra* allele both in diploid and in triploid forms. Dosage interactions in diploid and in triploids prove, according to the authors, that the + allele of the wild type is a sex gene like *tra* and *Hr* and that the three genes must be assigned to a single major locus for sex determination in the third chromosome.

Three genotypes appeared particularly interesting: (1) the *Hr*/*tra* diploid genotype with predominantly male gonads and male-like but retracted genitalia; (2) the *Hr/tra/tra* flies having elongated testes and predominantly male genitalia; (3) the *Hr/tra/+* genotype showing gonads of ovarian type but attached to an ejaculatory duct and rudimentary claspers and sex combs of five to six teeth only. The action of *Hr* appears therefore not so strong as that of *tra* and the gonads and genital ducts appear very variable in intersexes, that never attain complete sex reversal.

A similar high degree of variability was discovered by Lebedeff (1934, 1939) in diploid XX individuals of *Drosophila virilis* which are converted into intersexuals by a recessive gene located in the third chromosome.

Another case of intersexuality was discovered by Dobzhansky and Spassy (1941) in *Drosophila pseudoobscura* where a single dominant gene transforms diploid females into intersexes. They have only one pair of gonads but two more or less complete sets of genital ducts and external genitalia, one male and the other female-like. Sex combs are present in reduced number.

As Gowen and Fung justly pointed out the study of most single mutant genes enumerated above had not shown that the corresponding normal alleles operate in the sex differentiation. Dosage interactions have proved on the contrary, in Gowen and Fung's interpretation, that the wild type allele of the *Hr* and *tra* series is a sex gene and not a sex modifier, and therefore a major sex locus exists in the third chromosome of *Drosophila melanogaster*.

No contradiction exists with Pipkin's results since the wild type allele of the series studied by Gowen and Fung has not a strong male determining action, as proved by dosage experiments.

7. The Sex Ratio Problem in Drosophila

Marked deviation from the 1 : 1 sex ratio in *Drosophila*, which consist in the production of a totally or almost totally female offspring, are determined by two essentially different mechanisms.

In some cases, whose first example was first described by Morgan, Bridges and Sturtevant (1925) in *Drosophila affinis*, the males belonging to some strains gave an exclusively female offspring independently from the genetic make-up of their female partners. In other instances the factor for the production of a purely female offspring passes from mother to daughters exclusively and the few exceptional males were unable to reproduce the same effect in their progeny (Cavalcanti, 1950).

Perhaps the best-known example of sex ratio gene has been found by Sturtevant and Dobzhansky (1936) in wild populations of *Drosophila pseudoobscura* and in what has later been called *Drosophila persimilis*.

The gene is sex-linked and acts only in male individuals where no pairing takes place between the X and the Y during spermatogenesis and the X splits twice at diakinesis forming thus four chromatids instead of two. As the X divides at both meiotic division, each sperm carries an X and therefore they are all female determining. The Y does not divide and usually degenerates.

Only in a few instances some sperms receive no X chromosome and the Y is included in them. Less than 10 per cent of males are thus produced.

The gene is sex limited as it has effect in males only. It was ascertained later (Darlington and Dobzhansky, 1942) that the percentage of male progeny is higher at 25°C than at 16·5°C and it was suggested that the abnormal behaviour of the X is caused by its increased nucleinization as temperature was supposed to influence the amount of nucleic acid in the chromosomes. The resemblance of the temperature effect with the mechanism shown by Seiler in the *Talaeporia* case (see p. 84) is also evident and it might also account for the different sex ratio at different temperatures. A constant association of the sex ratio gene with inversions has also been evidenced by Dobzhansky (1939).

Gershenson also studied (1928) a similar sex ratio factor in *Drosophila obscura* and pointed out to the fact that a male bearer of the sex ratio gene transmits its X chromosome to the whole progeny while any other sex-linked gene is inherited by only one half of the progeny. As a consequence any population where the sex ratio gene appears should become homozygous for it, and it should consist of females with very few males, if any. The subsequent research by Sturtevant and Dobzhansky (1936) showed that the frequency of sex ratio varies from 0 to 40 per cent in different populations of *Drosophila pseudoobscura* but a frequency approaching 100 per cent has never been observed. The problem of the diffusion of such gene in nature has been recently discussed by Shaw (1958) and it will be dealt with in connection with sex ratio genes in isopods and in cyprinodont fishes (see Chapter 6).

A quite different type of inheritance of the sex ratio condition was discovered in *Drosophila prosaltans* and in *Drosophila bifasciata* (Magni, 1952) where it is inherited strictly through the female line and exceptional males produced in such lines do not differ from the male progeny of normal females in their influence on the sex proportions in their offspring. A study by Magni (1954) showed that the sex ratio condition is maintained even when all the chromosomes of the original sex ratio strain are replaced with homologous chromosomes coming from lines with normal sex ratio. This result was confirmed by Cavalcanti and Falcão (1954) in *Drosophila prosaltans* and by Malogolowkin in *Drosophila willistoni* and *Drosophila paulistorum* (1958).

The cytoplasmic nature of sex ratio factor in *Drosophila bifasciata* was shown by the results of crosses of sex ratio females with normal males at a culture temperature of 26°C which produced 133 male and 110 adult female individuals. Females born at 26°C breed like normal females (Magni, 1954) and such apparently irreversible loss of the sex ratio has been observed also in *Drosophila willistoni* and in *Drosophila paulistorum* (Malogolowkin, 1958). An injection of cytoplasmic material from abnormal eggs into the abdominal cavity of normal females induced them to produce after fertilization sons and daughters in normal ratio at first and only daughters later (Malogolowkin and Poulson, 1957).

The various investigators substantially agree that the plasmatic infective factor acts by killing the eggs fertilized by Y carrying sperms or containing only one X. An allelic pair *Sr*/*sr* of *Drosophila prosaltans* influences the activity of the cytoplasmic factor which becomes lost in the presence of the recessive *sr*/*sr* homozygote and reproduces normally only in the presence of the dominant *Sr* allele.

The resemblance of the sex ratio "cytoplasmic" sex ratio factor with the factor for sensitivity to CO_2 in *Drosophila* and especially to the *killer* factor in *Paramecium* are very impressive. The recent discovery by Poulson and Sakaguki (1961) that the maternally inherited sex ratio condition is produced in *Drosophila nebulosa*, in *D. willistoni* and in *D. melanogaster* by small spirochetes, which may be carried in latent condition, raises the interesting problem of the inter-relationship between spirochete and host genotypes.

Diploid Intersexes in *Lymantria dispar*

Research on sex determination in the gypsy moth *Lymantria dispar* is essentially based on inter-racial crosses that give rise to a rich and graduated series of intersexes (Fig. 5.2) which do not differ in chromosome number from the normal individuals.

The marked sex dimorphism between male and female moths makes possible, on the other hand, a detailed analysis of sex intergrades, the male being smaller and more slender than the female, which has a whitish colour while the male is dark brown. The antennae of the male are also markedly pectinate while the antennae of the female are almost thread-like.

The diploid number of chromosomes is sixty-two both in the homogametic male sex and in the digametic female sex where the XY (or WZ) group is cytologically not detectable.

Intersexual moths are diploid and have different strengths of the sex determiners in the various geographic races which live in the paleartic region. This made possible Goldschmidt's experiments (1915–1938) on the genetic analysis of sex determination. Chromosomes of different sizes were found in the different races (Saitoh, 1958).

Goldschmidt established that crosses within the numerous races of *Lymantria dispar* always form normal male and female individuals and that intersexes arise from inter-racial crosses.

Reciprocal crosses between European and Japanese moths gave the following results: from the cross J♂ × E♀ normal males and intersexual females are obtained. If female intersexes are not sterile the F_2 individuals are normal males and half normal and half intersexual females; the reciprocal cross E♂ × J♀ produces normal males and females in the F_1 generation but in the

F_2 generation normal females and half normal and half intersexual males are obtained (Fig. 5.3).

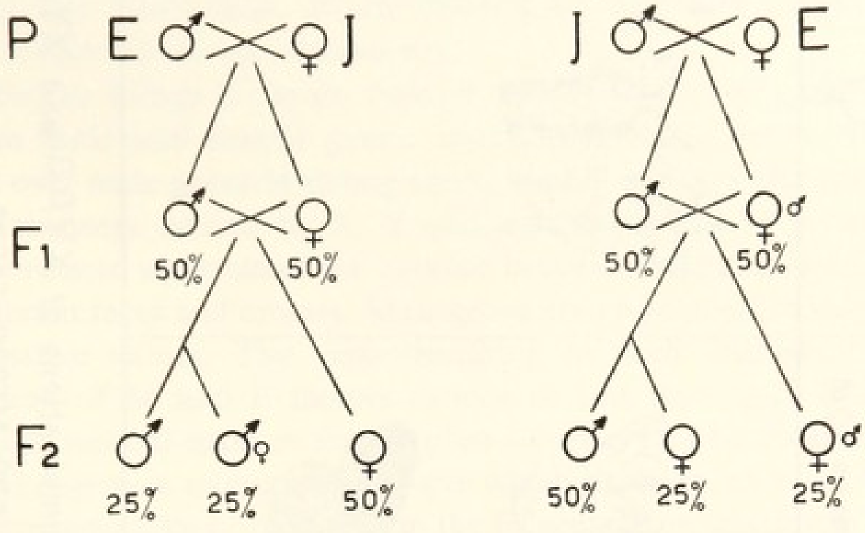

Fig. 5.3. Intersexes in reciprocal crosses between the European and Japanese races of *Lymantria dispar* (after Goldschmidt).

Intersexes are distinguished in male and female intersexes: the intersexual XX moths with various degrees of female characters are the male intersexes and female intersexes the others. Complete sex reversal has been observed in certain crosses and series of various degrees of intersexuality can be made (Fig. 5.4).

An interpretation of the above results has been obtained by assuming that the absolute values (or valences) of the M and F factors are such that in inter-racial crosses the M factors always prevail upon the F factors in the XX individuals and the F upon the M factors in the XY individuals. Intersexes are produced in inter-racial crosses because the M and F valences are different in the different races.

The race whose males produce intersexual females in F_1, when crossed with females from another race, is the strongest and the Japanese race is the stronger, the European the weaker race in the case exemplified in the above diagram. Therefore in Goldschmidt's interpretation both male and female factors of the Japanese race possess a higher valence than the corresponding factors of the European race.

The quantitative relationship existing between F and M valences is well defined within each race and the difference between them always reaches a threshold which permits the normal development of male and female characters in XX and XY individuals. This limiting value, or threshold, below which intersexes are originated, is called the epistatic minimum.

This interpretation is essentially similar to the interpretation which was later advanced for *Drosophila* although in his pioneer work Goldschmidt

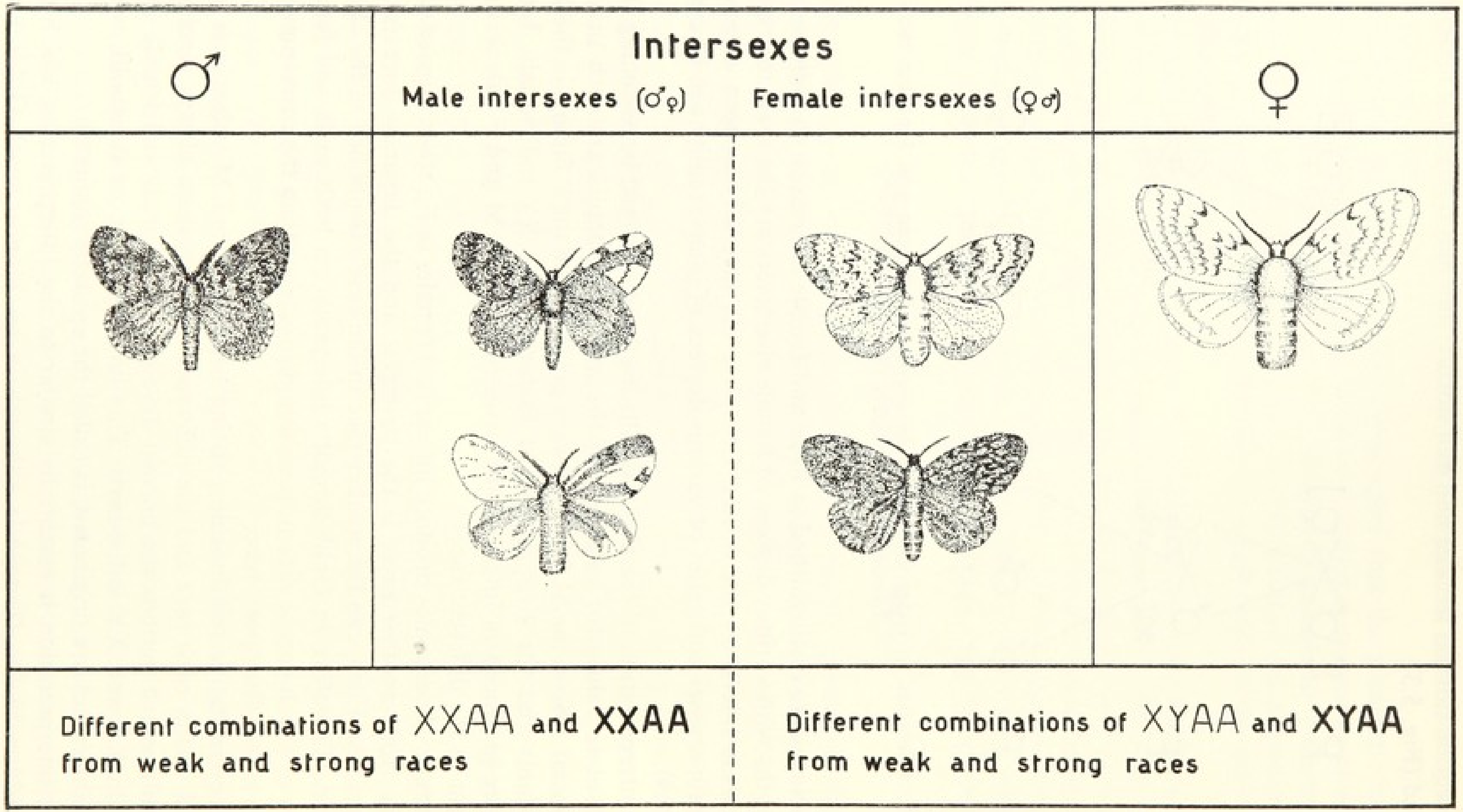

FIG. 5.4. Normal male and female individuals and male and female intersexes in *Lymantria dispar* (after Goldschmidt).

held for many years the opinion that the X chromosome of the gipsy moth carries male determiners and the cytoplasm carries female determiners. Goldschmidt (1942–1955) later accepted most of Winge's (1937) conclusions concerning the localization of sex determiners although he excluded the influence of autosomal sex determiners.

According to Winge X carries male, Y female determiners and autosomes carry both male and female genes: autosomal female genes are strongly prevalent over male genes in strong races, weakly prevalent in weak races.

Sex determiners carried by X, Y and autosomes can be given arbitrary values in order to understand the balance between male and female valences in the different races and crosses. Male genes are given positive values, female genes negative values. The value resulting in each individual from the algebric sum of M and F factors cannot be less than ±20 (the epistatic minimum) if normal male or female phenotypes are to be obtained.

Figure 5.5 gives a numerical example which explains, in accordance with the chromosome theory, the results in the F_1 generation which was illustrated in Fig. 5.3.

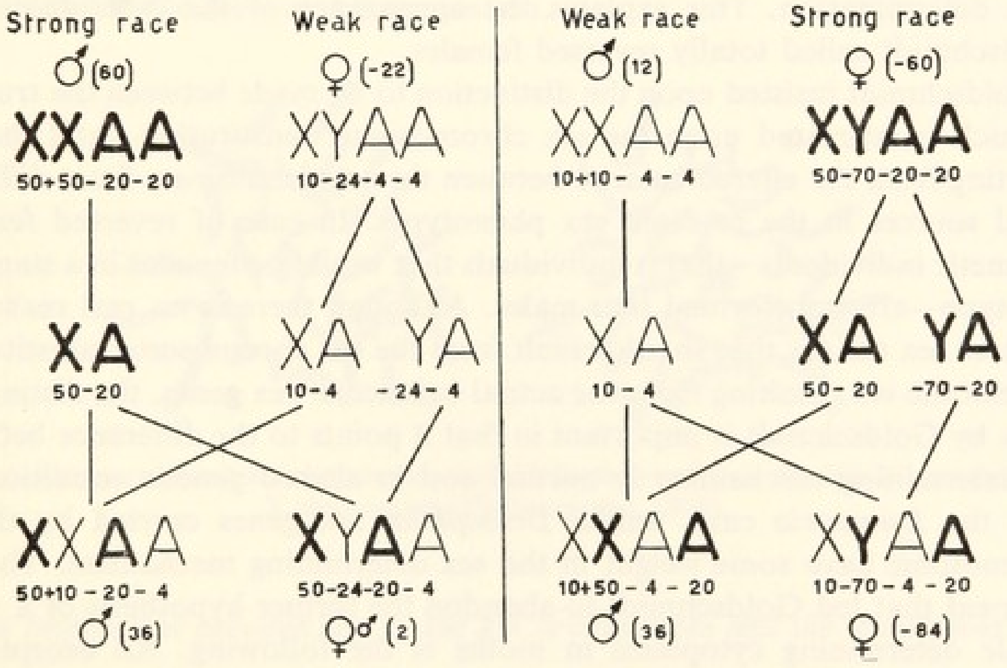

FIG. 5.5. Reciprocal crosses between a strong and a weak race of *Lymantria dispar* in Winge's interpretation: a balance in the action of heterochromosomal as well as of autosomal sex genes is involved (Winge, 1937).

It is conventionally assumed that the value of X in a strong race is five times that of a weak race. The genes for femaleness in Y have the valence of —74 in the Japanese and —24 in the European female. A haploid set of autosomes shows a prevalence of female genes which is valued —20 in the strong and —4 in the weak race.

Valences in the weak races are thus the following:

$$\male = XXAA = 10 + 10 - 4 - 4 = + 12$$
$$\female = XYAA = 10 - 24 - 4 - 4 = - 22$$

and in the strong race:

$$\male = XXAA = 50 + 50 - 20 - 20 = + 60$$
$$\female = XYAA = 50 - 70 - 20 - 20 = - 60$$

The diagram shows that the XY offspring produced by a cross of a European ♀ × Japanese ♂ has a value of + 2 and is therefore a female intersex, because it has the chromosomal formula of a female, with a slight predominance of male factors. Results of crosses in the next generation are not exemplified in the diagram but they can easily be obtained in the same way.

It has been shown by Goldschmidt that crosses of very strong males from races of Northern Japanese islands with weak females give rise to totally male offspring in the F_1 generations: if the values given for the strong race are multiplied three times and the values for the weak race are left unchanged in the offspring of a very strong male crossed with a weak female X and Y carrying individuals are obtained whose value is + 62, which means a strong male determination. This explains the appearance of the individuals that Goldschmidt called totally reversed females.

Goldschmidt insisted upon the distinction to be made between the true sex —which is estimated upon the sex chromosome constitution—and the sex resulting from the altered balance between the sex determiners from different racial sources in the reversed sex phenotypes. In case of reversed females digametic individuals—that is individuals that would be females in a standard genotype—are transformed into males. Although there is no real reason to call true sex the sex that should result from the sex chromosome constitution and not the sex resulting from the actual balance of sex genes, the distinction made by Goldschmidt is important in that it points to the difference between sex determining mechanism in normal and in altered genetic conditions.

In the *Lymantria* case, unlike *Drosophila*, sex genes carried by the Y chromosome have some weight in the sex determining mechanism. The experiment that led Goldschmidt to abandon the former hypothesis of a weak female determining cytoplasm in moths is the following. An exceptional Japanese male *Lymantria* carries a Y chromosome and is crossed with a normal European female that, according to the former hypothesis, should have a weak female determining cytoplasm. Despite the weak cytoplasm some strong females were obtained and their high valence could only be explained by assuming a female determining influence of the Y from the strong Japanese individual. This explanation is quite consistent with the results obtained in *Melandrium*, a flowering plant, where ample evidence for the sex determining influence of Y was found.

In the absence of detailed cytological evidence the picture of the system of sex determination in *Lymantria* may appear less clear than in *Drosophila* and the conventional values given sex genes in different races convey less striking evidence than the repetition of chromosomal units in *Drosophila* experiments. The concept of valence of sex determiners is nevertheless in agreement with the results in *Drosophila*. The dosage of male and female determining factors through crosses between races of different strengths allowed also a developmental analysis of the sex differentiating processes.

The *Melandrium* Case and the Sexual Function of Y

The most striking demonstration that Y contains sex determining genes in certain species was obtained as a result of extensive researches on *Melandrium album*, a seed plant of the pink family (Caryophyllaceae). *Melandrium album* and *Melandrium rubrum* are the only dioecious members of the group and the other species are monoecious. Winge and Blackburn found independently and in the same year (1923) the first example of sex chromosomes of angiosperms in *Melandrium album*. It is now certain (Westergaard, 1958) that the Y chromosome is the largest member of the pair (Fig. 5.6) according to the original Blackburn's interpretation.

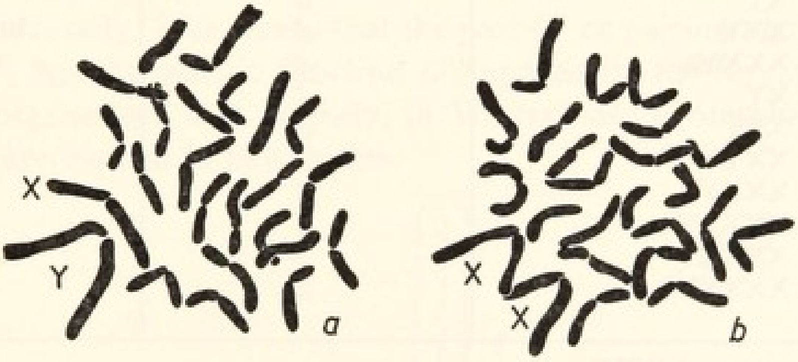

FIG. 5.6. Autosomes and sex chromosomes of *Melandrium album* : the Y is bigger than the X chromosome (Westergaard, 1948).

1. The Interaction between the Y, the X Chromosomes and the Autosomes

Independent researches by Warmke (1939–1946), by Ohno (1939) and by Westergaard (1940–1959) first established that sex is not determined, as in *Drosophila* and in *Rumex*, by the ratio of X and autosomes, but is induced mainly, if not exclusively, by the interaction of the genes in the X and in the Y chromosomes. Normal females have two X and plants with two X and four sets of autosomes (instead of the normal two) are female. Plants with two X's and a Y develop into males and a single Y can suppress the female potentials of three X chromosomes in individuals with four sets of autosomes. Only when the ratio is shifted to Y/4X, plants are bisexual in most flowers of

Warmke's strains and they are mostly pure male or only slightly hermaphrodite in Westergaard strains. The Y is thus in *Melandrium* much more potent in the direction of maleness than X is in the direction of femaleness as conclusively shown in the table by Westergaard (1953) (Table 2) where Warmke's results are indicated by a cross.

TABLE 2. THE RELATION BETWEEN CHROMOSOME CONSTITUTION AND SEX IN *Melandrium* AND *Drosophila**

Chromosome constitution	*Melandrium*	*Drosophila*
1. 2A + XX	♀	♀
2. 2A + XXX	♀	♀
3. 3A + X	—	♂
4. 3A + XX	♀	Intersex
5. 3A + XXX	♀	♀
6. 4A + XX	♀	♂
7. 4A + XXX	♀	Intersex
8. 4A + XXXX	♀	♀
9. 4A + XXXXX	♀	—
10. 2A + XY	♂	♂
11. 2A + XYY	♂	♂
12. 2A + XXY	♂	♀
13. 2A + XXYY	—	♀
14. 3A + XY	♂	—
15. 3A + XXY	♂	Intersex
16. 3A + XXXY	♂	♀
17. 4A + XY	♂	—
18. 4A + XXY	♂	—
19. 4A + XXYY	♂	—
20. 4A + XXXY	♂	—
21. 4A + XXXYY	♂	—
22. 4A + XXXXY	♂→ ♀	—
23. 4A + XXXXYY	♂	—

* From Westergaard (1953)

Table 2 might lead to the wrong conclusion that autosomes of *Melandrium* take no part in the sex balance. Westergaard's results in 1948 demonstrated, on the contrary, a weak influence of autosomal sex determiners. Andromonoecious plants were obtained, in addition to female and male plants, from crosses between diploids. These exceptional plants have a Y chromosome and three sets of autosomes. The first flowers are pure hermaphrodite, the next ones are faintly hermaphrodite and the last are male. Self-fertilization of the androhermaphrodites made possible, through selection, the establishment of real hermaphrodite plants which showed only hermaphrodite flowers.

These results lead to the conclusion that some autosomal combinations can have a feminizing influence since androhermaphrodites (weakly male hermaphrodites) and real hermaphrodites have identical constitution in regard to sex chromosomes.

The sex balance in *Melandrium album* is thus the result of a strong male action of sex determiners in the Y chromosome, and of a less strong action of the female sex determiners in the X chromosome which can be enhanced by certain combinations of autosomes.

2. *Experiments with Fragments of Sex Chromosomes*

Studies on sex expression in plants with fragmented Y chromosomes allowed Westergaard to localize gene complexes with different functions within the sex chromosomes. According to this author two types of sex genes exist, namely the sex deciding genes which are confined to the sex chromosomes (X or Y or both) and the basic sex genes in both autosomes and sex chromosomes: their interaction determines which sex will be expressed. Such view (which recalls the Correns–Hartmann's theory of the AG complex and sex realizators) will be discussed in a later chapter. The present report is therefore confined to the results concerning the localization of gene complexes and their interaction in the X and Y chromosomes.

Figure 5.7 shows the segments into which Westergaard was able to divide the sex chromosomes. Regions I–II–III form the differential segment of Y and correspond to the fragmented chromosome that has been indicated as Y^1. Plants with such fragments only are hermaphrodites, that is they contain bisexual flowers only. This means that the part IV or pairing segment, which is lost in Y^1, has the genetic function of suppressing the formation of the female sex organs and it is unlikely, in Westergaard's interpretation, that other genes are located in that region.

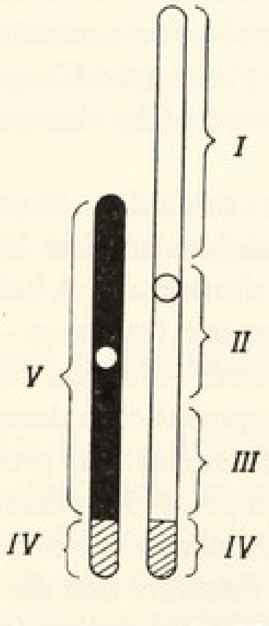

FIG. 5.7. A diagram of the sex chromosomes in *Melandrium*: I, female "suppressor" segment where are located genes that suppress the formation of female sex organs; II, male "promotor" section which contains genes that control the initial anther development; III, the segment controlling the last stages of anther development; IV, the pairing segments; V, the differential segments of X (Westergaard, 1953).

The differential region and part of the pairing region (segments III–IV) are absent in the Y^3 chromosome and in their absence pollen mother cells degenerate and thus plants with Y^3, instead of Y, are male sterile: segment III controls thus the last stages of anther development. Similarly segment I contains a female suppressor gene (or genes) because, if that part is absent, a normal bisexual part is formed. Segment II contains a gene (or genes) which initiates anther development since the absence of the whole Y chromosome results in the formation of a female plant.

In Westergaard's picture sex is determined by Y through a trigger mechanism which is brought about through complete linkage between genes that suppress female development and genes which initiate and complete anther development. This interpretation suggests therefore an evolution from a bisexual condition to unisexual condition through the development of a trigger mechanism which suppresses the potentialities of the opposite sex in males and in females.

Goldschmidt (1955) has given a thorough discussion of Westergaard's work on *Melandrium* and reached the conclusion that *Melandrium* has the same type of sex determination as *Drosophila* plus a strong antifemale modifying action of the Y heterochromatin because the right section of Y doesn't actually have—according to him—a function in sex determination but only a control on the maturation of already determined male cells.

It is difficult, in the present author's opinion, to decide in many instances which are and which are not to be considered as sex factors but it is quite evident that dioecism represents in the genus *Melandrium* a recent acquisition which has been obtained through rather simple and crude mechanisms. The action of the tassel seed and silkless mutants, which have been employed in obtaining dioecious maize plants (see Chapter 8), actually appears to be very similar to the influence exerted by the sections of sex chromosomes in the *Melandrium* system.

Smith (1963) has recently studied a North Carolina race of *Rumex hastatulus* which is usually dioecious and has XY_1Y_2 males ($2n = 9$) and XX females ($2n = 8$). The species showed a X/A balance in sex determination but Smith demonstrated that a centric fragment—which is capable of chiasma formation with a Y chromosome—and has been named the Y fragment—contains a factor for the development of maleness. The evidence was given by $2A + XX$ plants plus the Y fragment that produced hermaphrodite flowers and by two triploid plants with $3A + 3X$ plus the same fragment which were intersexes although $3A + 3X$ plants develop as normal females. The influence of the fragment shows therefore that sex is determined in *R. hastatulus* by a X/A plus Y system which is intermediate between the *Drosophila* and the *Melandrium* systems.

Comparable results (summarized by Kihara, 1953), were obtained earlier by Tazima (1943) in the silkworm *Bombyx mori*, which shows female digamety, like all other Lepidoptera. Neither intersexes nor X-linked characters were found in the course of experiments and it was shown that all individuals are males in the absence of the Y (= W) chromosome regardless of the number of doses of autosomes or of X (= Z) chromosomes. The presence of Y always determined the female sex and experiments with different fragments of Y showed that the factors for female determination are confined to a limited section of the Y chromosome.

The work on *Bombyx* as well as the more detailed work on *Melandrium* provided thus the first conclusive evidence regarding the existence of male (or female) sex determiners in the Y chromosome. They provided a useful model for a number of other organisms, including mammals. Yamamoto also demonstrated a male determining property of the Y chromosome in the Cyprinodont fish *Oryzias latipes* as a fertile XXY male was obtained (1963). The Y chromosome is constituted by a so-called inert segment in which major genes are thought to be deteriorated or absent, by the homologous segment including the *r* locus and by the differential segment. The inert section of Y would be sheltered from natural selection, in Yamamoto interpretation (1964), by a corresponding viability section in the X.

With regard to the system of suppressor regions that Westergaard has discovered in the sex chromosomes of *Melandrium*, it is difficult to judge at present its value as a general model for sex determination.

Sex Determining Mechanisms in Mammals, Including Man

The application of the technique of tissue culture to the study of human chromosomes, the discovery of the so-called sex chromatin (Barr, 1955) and the demonstration that the Y chromosome is the bearer of male determining factors in the mouse (Welshons and Russell, 1959) have recently stimulated a great deal of work on the problem of sex determination in mammals and especially in man.

Cytological investigation showed early the existence of well differentiated sex chromosomes in mammals: both Marsupials and Placentals possess an XY pair in the male sex.

1. The Chromosome Complement in Normal Humans and their Relatives, in Intersexes and in Gynanders

Researches by Koller and Darlington (1934) on meiotic chromosomes in rats indicated that the differential segments include the centromeres and a true synapsis takes place between the terminal homologous segments. The work of Sachs (1954) on human sex chromosomes at meiosis showed that they are included on a sex vesicle at pachytene without forming any real chiasmata. Recent work indicates (Ferguson-Smith, 1964) that primary spermatocytes possess three long and two short bivalents that form terminal nucleoli. The study of chromomere patterns has just begun.

Most recent information on human chromosomes derives, however, from metaphase plates obtained from tissue cultures, a method that supplies very clear pictures, although it does not provide as much information as the study of meiotic phases. The X chromosome of men appears medium sized, with a submedian centromere and Y appears very short and acrocentric in preparations from mitotic metaphases (Pl. II). It has been established only in recent years that the diploid number of human chromosomes is forty-six when Tijo and Levan (1956) obtained excellent preparations from tissue cultures of embryonic lung cells. Their result was soon confirmed by Ford and Hamerton (1956) who found twenty-three bivalents in the first meiotic division of spermatocytes.

Subsequent work by various cytologists has led to an agreement concerning a standard system of nomenclature of the human mitotic chromosomes and the caryotype of the normal human male (Pl. II) where the twenty-two autosomes have been classified into seven groups (The Human Chromosome Study Group or the Denver Statement, 1960). The alleged occurrence of supernumerary chromosomes in normal individuals, has been ruled out by the work of Makino and Sasaki (1960).

Recent studies on the chromosomes of the nearest relatives to man have established that orang utan, gorilla and chimpanzee have $2n = 48$ chromosomes (Chiarelli, 1961, 1962; Hamerton *et al.*, 1961). Comparative analysis has led us to suppose that a fusion of two acrocentric chromosomes in early man has given rise to either chromosome 2 or chromosome 5 of the Denver nomenclature since polysomy is not likely to have occurred in the evolution of the anthropoids (Chiarelli, 1962). The X chromosome of the orang utan is very similar to that of the man and the Y chromosome is always very small both in apes and in man (Pl. II).

Lejeune, Turpin and Gauthier discovered in 1959 that individuals affected by mongolian idiocy are trisomics for a small acrocentric autosome, which has been identified with chromosome 21 (or 22) of the Denver system. Their finding was soon confirmed by a number of other investigators who found the same chromosome presented in triplicate in several cases of mongolism (Hayashi, 1963).

Some abnormal sexual syndromes in man have been correlated with abnormal numbers of sex chromosomes and their analysis has been facilitated by the discovery of the so-called sex chromatin.

A basophilic mass was discovered by Barr and Bertrame (1949) in the interphase nuclei of nerve cells of female cats and proved to be absent in nerve cells nuclei of at least a vast majority of the males. Such mass resulted later to be Feulgen positive. Moore and Barr (1954) found the sex chromatin in the woman and a number of investigations has shown the existence of the so-called sex chromatin (or Barr's body) in the females of several mammalian species.

It was early assumed that the sex chromatin is produced by the presence of two X chromosomes and possibly by their pairing in the digametic sex. Later observations by Ohno (1961) both on rodents and in man brought, however, some strong evidence that sex chromatin is the expression of a single heterochromatic X chromosome. This has been the starting point of an important hypothesis by Lyon (1961, 1962) which will be briefly mentioned after a discussion on some sexual syndromes and their connexion to alterations in the number of sex chromosomes.

Male individuals affected by the so-called Klinefelter's syndrome show small testes which usually do not produce ripe sperms and often exhibit an abnormal development of the mammary glands. In several such cases Ford and co-workers (1959) discovered the existence of XXY chromosomes (47 chromosomes) and the search for sex chromatin showed the existence of chromatin positive nuclei in the majority of patients affected by such syndrome. Mosaicism (see p. 83) is also frequent in individuals affected by the Klinefelter's syndrome: Ford and collaborators (1959c) found an XXY/XX and Buckton *et al.* (1961) XXY/XY mosaic in such patients. They were sex chromatin positive with a single Barr's body.

A similar discovery was made also by Ford *et al.* (1959a) in patients affected by the Turner's syndrome. They are essentially female phenotypes showing rudimentary ovaries or no ovaries at all and retaining an infantile development of the mammary gland and other parts. Most of them show an XO sex chromosome constitution and are sex chromatin negative. Some individuals affected by the Turner's syndrome turned out to be XO/XX mosaics with a single Barr's body (Lindsten, 1963) or XO/XXX mosaics with two Barr's bodies (Jacobs *et al.*, 1960).

An oligophrenic female with five X chromosomes showed four Barr's bodies (Grumbach *et al.*, 1963).

Mental deficiency and undescended testes have been observed in various XXYY males and in two males with XYY sex chromosomes and no sex chromatin (Fraccaro *et al.*, 1962). An extreme example of male determining influence of the Y chromosome in man is represented by males with XXXXY chromosomes and three Barr's bodies. They show in general undescended testes, radio ulnar synostosis and mental deficiency (Fraser *et al.*, 1962; Fraccaro *et al.*, 1962). As Lyon's theory (see p. 111) is likely to apply, at least partially, to man such instances are not, however, to be confused with similar cases in *Melandrium*.

In sex mosaics the Y chromosome has not apparently exerted so strong influence in the male direction: a woman with clinical evidence of masculinization turned out to be a XX/XXY/XXYYY mosaic (Fraccaro *et al.*, 1962) and a patient affected by the so-called true hermaphroditism was found to be a XX/XXY mosaic (Turpin *et al.*, 1962).

The patients that show testicular and ovarian tissues, both non-functional, are called true hermaphrodites in most papers on the clinical aspects of sex abnormalities and the patients whose abnormalities regard only external genitalia and secondary sex characters are named pseudohermaphrodites. The XY pair and sex chromatin negative nuclei have been found in so-called male hermaphrodites (they have male type gonads) and the XX pair of chromosomes with sex chromatin positive nuclei in the female hermaphrodites. Low degrees of intersexuality can thus be shown in man also in the absence of alterations of the normal number of sex chromosomes and also in the absence of tumours which may greatly influence the sex phenotype.

A certain confusion is originated by the different names given to some abnormal sex phenotypes in the general biological literature and in the medical literature where such terms as intersexuality or sex mosaicism have been introduced only very recently (see Overzier, 1958) and seem to be accepted with some reluctance.

The patients with ovaries and testes are still commonly indicated as true hermaphrodites, or with mixed external genitalia as pseudohermaphrodites, the individuals affected by the Turner's or by the Klinefelter's syndromes are strong or weak intersexes and it should not be difficult to devise a system of nomenclature in which basic genetical data could be integrated with clinical information. The increasing number of sex mosaics which are being presently discovered will undoubtedly render the task much more difficult for intersex-like gynanders, which on the other hand propose fascinating problems of sex differentiation.

The mechanism of the sex balance in man is on the other hand not very difficult to understand in its broadest implications. The XO constitution of most patients affected by the Turner's syndrome, the XXY constitutions of male type intersexes, the XXXXY male phenotypes and a number of other sex chromosome combinations in abnormal sex phenotypes point to a balance between the male determining influence of the Y chromosome and a female determining action of X.

Differential frequencies on the nuclear types that form the sex mosaics at different stages of their development appear to be responsible for considerable variations in the male expression of Y carrying mosaics. And very little can be said about the balance between the X and the Y chromosomes until it will be established whether only one X chromosome is genetically active in man.

Examples of gonadal disgenesis and similar abnormalities that show the normal male or female chromosome complement suggest also the possibility of sex determining influences of the autosomal set such as have been demonstrated in the *Melandrium* experiments and especially by Winge in his work on *Lebistes* (see Chapter 6). Peculiar disturbances of the sex balance due to autosomal factors appear highly probable in *Homo sapiens*, a species which shows an extremely high degree of genetic polymorphism.

Degenerative modifications of the egg or the early germ line may both cause chromosomal aberrations or be the direct cause of gonadal disgenesis as it has been pointed out by Witschi (1960). The developmental analysis of vertebrate sex differentiation (see Chapter 9) supplies ample proofs in favour of such possibility but genetic analysis is also contributing increasing evidence for the influence of autosomal sex genes or modifiers. Sex differentiation makes no exception for the human sex phenotype in being the result of integrated genetic and environmental (or postgenetic) influences.

2. XO and XXY Mice: Lyon's Hypothesis

Cytological observations showed that in the mouse some exceptional females have thirty-nine chromosomes as compared to forty found in normal XX females. Genetic experiments utilizing the mutations *scurfy* and *Tabby* as sex-linked markers demonstrated, on the other hand, that these exceptional females are hemizygous for the X chromosomes and therefore they have an XO constitution. They are fertile unlike the XO human patients that are affected by the Turner's syndrome and therefore a single X chromosome is sufficient in female mice for a normal development.

Welshons and Russell (1959) concluded from their experiments that the Y chromosome is male determining. Cattanach (1961) complemented the experiments by obtaining XXY sterile males that carried a marked X chromosome of paternal origin and demonstrated that XO females are developed by non-disjunction in the male parent.

It had been demonstrated by a series of previous researches that female mice heterozygous for X linked colour genes constantly show a variegated, or mosaic phenotype with zones of mutant as well as wild-type coat colours: the XXY individuals of Cattenach's experiments showed such variegation since they carried a wild type-allele of chinchilla attached to their X chromosome of paternal origin and were thus heterozygous for genes that had become sex-linked through translocation.

Such observations were correlated with the already mentioned data that in female somatic cell one X is heterochromatic forming a Barr's body, and one is isochromatic and that in male somatic cells the single X chromosome is isochromatic (see p. 11).

Lyon (1961) assumed therefore that: (1) both normal male and female mice have only one functional X in their somatic cells and that the genetically inactive X forms the Barr's body; (2) the inactivation of one of the X chromosomes takes place at an early developmental stage and, once it has taken place in a cell, it is irreversible and it is transmitted to all its lineage; (3) the body of the normal female is therefore a mosaic of two cell populations with the one or the other inactivated X.

Ohno and Cattanach (1962) showed that the translocated portion of autosome on X becomes uniformly heteropycnotic in mice and behaves as an integral part of X according to an extension of Lyon's hypothesis.

Experiments are now in progress to investigate Lyon's theory especially in man. It has been shown in the previous chapter that the number of Barr's bodies corresponds in abnormal caryotypes to the total number of X chromosomes minus one. On the other hand XO, XX, XXX and XXXX individuals have not the same phenotype as they should have if all the X were inactivated but one. Bentler *et al.* (1962) reported that women heterozygous for a certain enzyme deficiency have two populations of erythrocytes which appear to have either a normal or a deficient enzyme level. It appears at present that, although Lyon's hypothesis has been wholly confirmed in mice, it is lacking complete evidence in man, where portions of X chromosome remain probably still active, as suggested by Russell (1963). The well-established difference between the *Drosophila* and the mammalian X, which behaves in two entirely different ways seems, however, to be the most promising contribution from the point of view of the action of sex chromosomes in mammals.

The Haplophase Sex in *Sphaerocarpus*

Researches by Douin (1909), which were later confirmed by Lorbeer (1927), demonstrated that in *Sphaerocarpus* male and female determining factors are segregated in one of the meiotic divisions since in each spore tetrad, which is surrounded by a common membrane, two of the spores result to produce male gametophytes and two female gametophytes.

Researches by Allen (1917) demonstrated the existence in *Sphaerocarpus donnelli* of a big X chromosome in the female gametophyte and of a much smaller Y chromosome in the male gametophyte. They are both present in the sporophyte and again segregate during the spore formation. The great differences in size between X and Y chromosomes are often correlated with the considerable size differences existing between female and male plants in several species of Musci (Fig. 5.8) (Lorbeer, 1927).

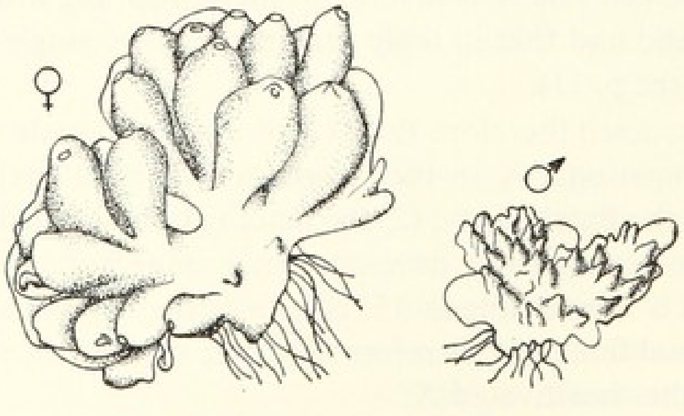

FIG. 5.8. A male and a female plant of *Sphaerocarpus texanus* (Allen, 1919).

Diploid gametophytes could not be obtained with Marchal's method in *Sphaerocarpus*: they are sometimes obtained through fusion of two haploid nuclei after the second meiotic division in spore formation. Such diploid spores (14 n + X + Y) gave mostly female gametophytes (Lorbeer, 1927) although Allen (1935) obtained hermaphrodite and female individuals in the course of his experiments on *Sphaerocarpus donnelli.* The prevalence of a unisexual condition, which is observed in the sporophyte of *Sphaerocarpus* as in the diploid gametophytes of Musci, is accompanied by a considerable sex variability.

The later work by Knapp (1935, 1939) and by Lorbeer (1936, 1938) also on *Sphaerocarpus donnelli* lead to very interesting conclusions on the sex in the haploid phase although some problems have still been left unsolved.

X-raying of spores and vegetation points of female gametophytes led to the production, both in Knapp's and in Lorbeer's experiments, of a number of male gametophytes. Knapp (1935) remarked that reversal from female to male is accompanied by the loss of a piece of the X chromosome and in a later paper (Knapp and Hoffmann, 1939) conclusively demonstrated that deletion of the X chromosome is not necessarily lethal, as contended by Lorbeer, and that, although even a very small deficiency is sufficient to induce reversal to the male sex in some regenerates, the number of male plants increases with the increase in size of the deficiency. These results can be explained with the alteration of the balance between the female factors in the X chromosome and male factors in the autosomes: the greater the deficiency, the higher the possibility of eliminating female factors from the X chromosome.

Certain experiments by Lorbeer suggest, however, the possibility of a different explanation: Lorbeer obtained monoecious individuals with 2X chromosomes. The so-called A mutant gametophytes develop antheridia and archegonia and they show a big fragment and a small spherical fragment of X: the B mutant gametophytes show only antheridia and no archegonia and cytological research demonstrates that only the big fragment of X is present.

Lorbeer deduced that the big fragment of X is male determining and that the small spherical fragment is female determining. It was concluded therefore that male realizators in the big fragment are the result of a mutation from female to male genes and that the spherical fragment of X contains the original female genes.

A monoecious C mutant was also obtained from the sterile clone of mutant B. This mutant showed male organs with unmotile spermatozoids in summer and normal archegonia in fall and in winter. A bigger and a smaller X chromosomes were present in such plants, the bigger probably corresponding to the masculinizing fragment of the B mutant the smaller being characterized by a marked bend in one arm. Fertilization of such plants by spermatozoids from normal male gametophytes originated a group of plants showing the same phenotypic and cytological characters of the mother plants and a group of gametophytes where the smaller X chromosome and a Y chromosome were present. A mutation from a male determining to hermaphrodite determining realizator was assumed by Lorbeer on the basis of this experiment although the above results can be explained as well with the simpler assumption that the smaller X is female determining.

Although some of the Lorbeer's conclusions appear rather puzzling his results seem to indicate that different segments of X contain genes or gene complexes with contrasting sex functions, like those illustrated by Westergaard in *Melandrium* and it is also likely that Knapp's results with fragments of X of different sizes represent the statistical results of such contrasting tendencies inside the X chromosomes.

Even if this hypothesis requires further verification it appears nevertheless quite sure, especially from Knapp's results, that sex balance in haploids works substantially in the same way as in the diploid organisms. Sex expression in *Sphaerocarpus* is clearly the result of a balance between the gene actions of female and male determining genes in the X chromosomes and in the autosomes.

Sexual formulae for haploid organisms have been proposed by Goldschmidt (1929): Mf stands for the male and MF for the female sex assuming that $F > M > f$. If M is located in the autosomes and F in the X chromosome the application to the case of *Sphaerocarpus* is quite convincing although it is not known yet the sexual function of Y in *Sphaerocarpus*. The questions arising from the application of the concept of the AG complex to sex determination in haploids will be discussed later.

CHAPTER 6

SEX DIGAMETY AND SEX POLYGAMETY IN GONOCHORIC POPULATIONS

THE genetic analysis of intersexuality in *Drosophila*, in *Melandrium* and in a few other organisms showed the existence of more or less intricate relationships between the male and female determiners located in the sex chromosomes and in the autosomes and demonstrated that the sex determiners carried by the X and by the Y chromosomes can be considered as the "sex deciding" factors in the typical cases of sex digamety.

The work of Winge (1923–1947) on the cyprinodont fish *Lebistes reticulatus* first indicated the existence of a sex determining system where the sex chromosomes are little differentiated and autosomal genes can assume prevalent importance in the determination of sex.

The later work of Kosswig and his collaborators (1930–1959) has gradually led a step farther, that is to demonstrate that no sex chromosomes exist in *Xiphophorus helleri*, another gonochoric cyprinodont fish, whose sex is determined through the balance between a number of male and female autosomal genes. Recent researches have demonstrated the widespread occurrence of similar types of sex determination among gonochoric organisms.

The so-called polyfactorial sex determination leads to the segregation of several kinds of gametes having different sex potencies and therefore *Xiphophorus helleri* shows what may be named sex poligamety or plurigamety (Bacci, 1961b).

Little data concerning the early evolution of the sex chromosome mechanism and attempts of experimental alteration of autosomes into sex chromosomes provide information regarding the relationships between the types of sex determining mechanisms resulting in sex plurigamety or in sex digamety.

Sex Determining Mechanism in Cyprinodont Fishes

The first order of Cyprinodontiformes includes many small sized species that show a marked sex dimorphism and are easily bred in freshwater aquaria. For this reason they were largely employed in studies on sex determination. The relative importance of the differential and of the pairing segments in the evolution of sex chromosomes, the relationship between male and female digamety are among the problems for which the study of cyprinodont fishes has provided most valuable information. No morphological difference could be detected between sex chromosomes and autosomes in teleosts where many examples of hermaphroditism are also known (D'Ancona, 1949; Bacci and Razzauti, 1957).

1. The Lebistes *Case*

The common guppy of the aquarists, *Lebistes reticulatus,* shows sexual dimorphism in an extremely marked degree with a female of a dull colour, measuring up to 5 cm in length, and a male averaging 4 cm in length, provided with a gonopodium and displaying a brightly coloured livery whose patterns are extremely variable and are controlled by numerous genes. The study of sex-linked genes demonstrated the existence of male heterogamety in normal strains (Winge, 1922, 1934) although no cytological evidence for sex chromosomes is available (Winge, 1922).

Nine sex-linked characters are strictly limited to the paternal line and are transmitted exclusively from male to male individuals (Winge, 1934). Genes for such characters are obviously carried by the Y chromosome in its differential segment, that is where no crossing-over with X takes place. The gene *Maculatus* (Ma), which produces a black spot on the dorsal fin, is one of the absolutely Y linked genes and has proved a good marker of the Y chromosome.

Other characters are transmitted according to the mechanism of sex-linked inheritance and therefore they generally pass from one male to half the males of the second generation through the heterozygosy of the females. These characters are nevertheless shown also by a small percentage of males of the first generation and this means that there is a certain frequency of crossing-over between two segments of Y and X chromosomes. This discovery by Winge (1923) was the first proof of the existence of a pairing segment between X and Y chromosomes. *Coccineus* (Co), *Vitellinus* (Vi), *Tigrinus* (Ti) and *Luteus* (Lu) are among the partially X linked genes that are more frequently used as markers of the X chromosome. The males that carry such genes on the pairing segment of Y, transmit the corresponding characters to male sons along the male line only. They have become partially Y linked traits and they remain such until a new crossing-over causes a return to the normal X linked inheritance. Male heterogamety is thus demonstrated in *Lebistes reticulatus* by the absolute Y linkage of some genes and by the partial linkage of some others.

The pairing segment of the sex chromosomes appears to be in *Lebistes* much longer than in *Drosophila.* The *Lebistes* condition in this respect is therefore considered primitive as the elimination of crossing-over is of paramount importance in separating male and female sex genes and consequently in preserving a stability of sex ratios and sex expression in unisexual organisms.

A completely new line of research was started from the observation of a slight degree of intersexuality in two among Winge's strains: some females showed feeble male traits, that is formation of a gonopodium and patterns of male type coloration. These females were regularly homogametic as demonstrated by gene markers for X chromosome (*Coccineus*, *Vitellinus* in one strain, *Tigrinus*, *Luteus* in the other). In crossing the two strains, the Y chromosome being marked by the completely linked *Maculatus* gene, Winge obtained the following results:

$$\text{P}\,(♀)\; X_{\text{Co-Vi}}\; X_{\text{Co-Vi}} \quad \times \quad X_{\text{Ti-Lu}}\; Y_{\text{Ma}}\,(♂)$$

$$\text{F}_1\,(♀)\; X_{\text{Co-Vi}}\; X_{\text{Ti-Lu}} \qquad\qquad X_{\text{Co-Vi}}\; Y_{\text{Ma}}\,(♂)$$

in the vast majority of individuals with only three individuals possessing the Co – Vi and Ti – Lu colour spots but no Ma although they proved to be physiologically and morphologically males. The absence of the *Maculatus* trait, which is completely linked with Y, proves the absence of the Y chromosome in these males while the presence of both *Vitellinus-Coccineus* and *Tigrinus-Luteus* proves that these males carry two X chromosomes like the females of the same generation. Winge concluded therefore that, in the absence of Y, genes for maleness can only be carried by autosomes. An XX male individual is represented in Fig. 7.1 and, although it carries the sex chromosome complement which is peculiar to normal female genotypes, it is a genetic male because there is no reason to assimilate autosomal male determining factors with enviromnental factors and to assume that, at a certain stage of sex chromosome evolution, sex determining factors located in the heterochromosomes are essentially dissimilar from those located in the autosomes.

According to Winge's interpretation (1934) autosomal factors for masculinity and femininity are in equilibrium in standard races so that sex is determined by the distribution of the X and Y chromosomes. In exceptional strains (such as those marked by Co-Vi and Ti-Lu genes in the present experiments) a prevalence of male determining autosomal genes occurs, and selective crossing may lead to such an accumulation of male determining factors that the autosomal male determining become stronger than the female determining genes even when two X chromosomes are present. Back-crosses of XX males to their mother produced, after prolonged selection, offspring, all XX, whose sex ratio was 1 : 1. Any resemblance with previously analysed cases of crosses between individuals having reversed sex phenotype is thus ruled out.

The mother—according to Winge—had only one heterozygous autosomal pair and had a genetic constitution very similar to that of a male. As the Y chromosome has been eliminated from the system, a single autosomal pair has taken over the role of sex chromosomes. The females have thus become digametic and the males homogametic.

Winge's experiments showed thus for the first time that it is possible to change from male to female digamety within the same species and therefore that there is little difference between X and Y chromosomes and between sex chromosomes and autosomes in certain unisexual organisms. For this reason autosomes may easily take over the role of sex chromosomes and it is demonstrated that at this little differentiated stage of evolution sex chromosomes may not have a predominant role in sex determination.

It must be remarked, however, that the 1: 1 sex ratio which Winge obtained in these crosses between XX male and female *Lebistes*, could never be fully stabilized. Only the suppression of crossing-over in the neo-sex chromosomes could lead to such result. The selection for autosomal female determining genes also led to the production of XY females where the Y genes for maleness were overpowered by autosomal female determining genes. Concerning these females with the typical male heterochromosomes the same remark can be made as for XX males. Since their femaleness is the result of a balance between the sex genes in the sex chromosomes and in the autosomes, they can properly be considered as true females and not as reversed males, as they would if Goldschmidt's interpretation of the *Lymantria* case were applied.

Crossings of XY type females with normal males produced YY individuals which resulted to be normal males. The extension of the pairing segment thus allowed the survival of individuals lacking the X chromosome, a result which could not be obtained in *Drosophila* and gave the first demonstration (which was supported by the later experiments on *Melandrium*) of the male determining influence of the Y chromosome.

The results obtained in Winge's experiments are summarized in Fig. 6.1 and are of fundamental importance for the present development of research on sex determination. They need, nevertheless, a careful re-examination in some points as, for example, crosses between XX males and females yield a 1 : 1 ratio only in spring but in winter the offspring are often all females. A number of intersexes (though seldom well marked) has also appeared in Winge's crosses. This means, as Winge himself pointed out, that differences between sex genes located in the two types of chromosomes are rather small but it also means that the work of the alleged autosomal heterozygous sex genes is not very efficient. A co-operation of several autosomal pairs appears at present more probable than the prevalent action of a single pair in a type of determination where wide shifts of sex proportions are possible.

The great importance of Winge's results lies, however, in the discovery of the existence of many autosomal genes, both male and female determining, that can rule out the influence of the sex genes which are concentrated in the little differentiated sex chromosomes.

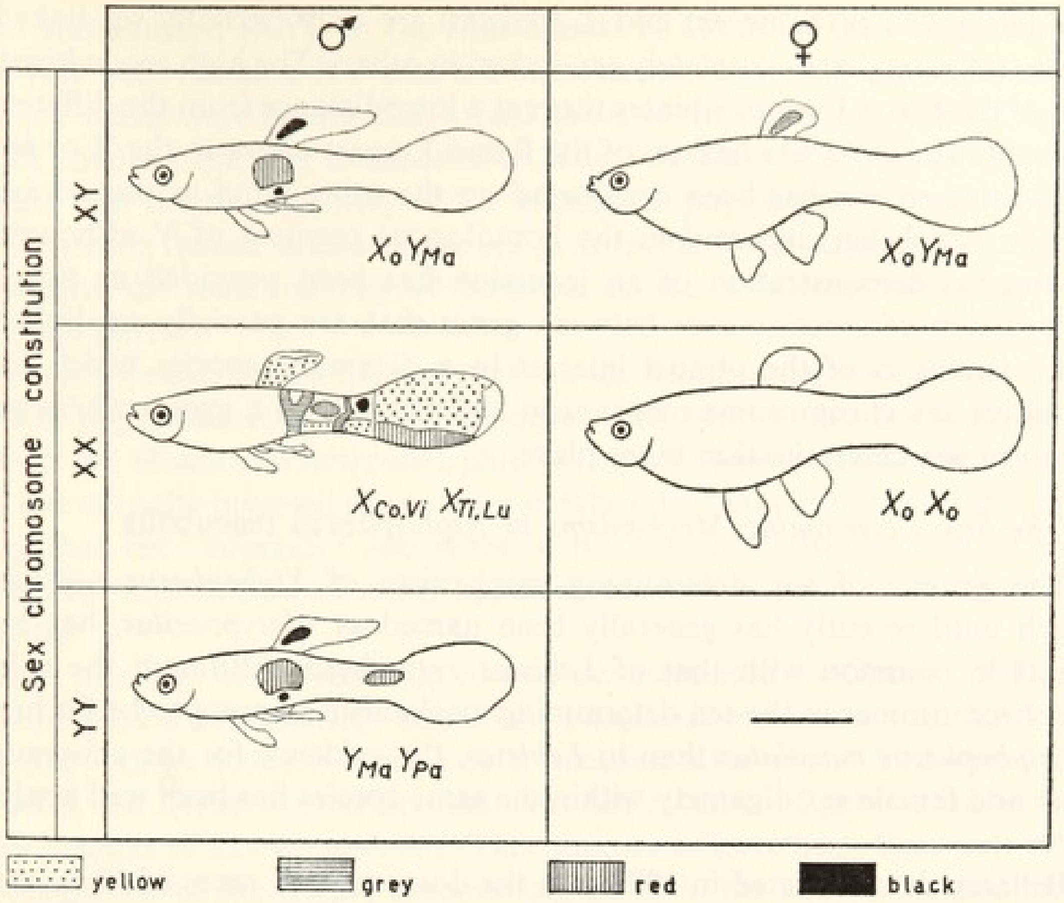

FIG. 6.1. XX males and XY females have been obtained in *Lebistes reticulatus* through selection of "minor" autosomal sex genes from strains which originally showed male digamety. Autosomal genes have thus become "sex deciding" genes. X and Y chromosomes are marked by completely (Ma) or by incompletely (Co, Vi, Ti, Lu, Pa) sex-linked genes (after Winge, 1934–1947).

The *Lebistes* type of sex determination is just inside the borderline separating sex digamety from sex polygamety.

This conclusion is supported by the observations of Spurway (1957) who established the occurrence of hermaphroditism with self-fertilization in superficially normal females having a XX constitution. Such individuals produced an all-female offspring with the exception of four males only. One of them was functional and had an XX constitution like the males of Winge's experiments.

It must now be remembered that examples of reversed sex phenotypes were shown in the marine Isopod *Idothea balthica* (see Chapter 4). Frequent sex reversal can be regarded in normally unisexual species as indications of a labile or primitive system of sex determination. According to the recent results of Tinturier Hamelin (1963) *I. balthica* is a female digametic species with a sex chromosome constitution closely resembling that of *Lebistes reticulatus*. The alleles of the gene F (*flavofusca*) are completely linked to the Y (−W) chromosome and are located therefore in its differential segment.

The two genes B (*bilineata*) and L (*lineata*) are only partially sex-linked in some strains and are completely sex-linked in others. The high recombination rate of the B and L genes situates them at a long distance from the differential segment. The complete linkage of the B and L genes either to the X or to the Y in some strains has been considered on the other hand as due to an inversion which has occurred in the homologous segment of Y although no cytological demonstration of an inversion has been provided as yet. The suppression of crossing-over between genes that are partially sex-linked in some strains is of the utmost interest in a digametic species which has a primitive sex chromosome mechanism and belongs to a group where polyfactorial sex determination takes place.

2. The Sex Determining Mechanisms in Xiphophorus maculatus

The genetic of sex determining mechanism of *Xiphophorus maculatus*, which until recently has generally been named as *Platypoecilus*, has many points in common with that of *Lebistes reticulatus*. Although the role of sex chromosomes in the sex determining mechanism appears to be far higher in *Xiphophorus maculatus* than in *Lebistes*, the evidence for the existence of male and female sex digamety within the same species has been well analysed here.

Bellamy demonstrated in 1922 that the domesticated races of *Xiphophorus maculatus* are female digametic and Gordon (1951) found the same mechanism in members of natural populations from the Belize River in British Honduras but wild strains from Mexican rivers revealed male digamety (Gordon 1946, 1947, 1951).

Exceptional males that appeared to be XY were also discovered in domesticated stocks (Breider, 1942; Bellamy and Queal, 1951) but they were interpreted as the results of early inversion since in the offspring of such males with normal females a sex ratio of three females to one male was obtained, like in the already illustrated instances of reversed sex phenotypes.

According to Gordon's analysis the pair of chromosomes responsible for sex determination is the same both in populations with male and in populations with female digamety and the difference appears to be due to a series of multiple alleles. The Y chromosome from the populations of British Honduras and Rio Jamapa in Mexico is male determining, and even more strongly male determining is Y' from Rio Coazacoalcos. The X chromosomes from both the Mexican rivers Coazacoalcos and Jamapa have a normal female influence while the corresponding chromosome element from British Honduras, which has been indicated by Gordon as the W chromosome, shows a stronger female influence.

Conclusions concerning what might be called the relative valences of sex alleles of the different populations could be drawn from the results of crosses between a digametic female from British Honduras (WY) and a digametic male from Rio Jamapa (XY). In the four classes of offspring thus obtained WY and WX were females, XY and YY were males. WX, WY and XX females and XY males were obtained in a back-cross of the new class of WX females to XY' males from Rio Coazacoalcos. The 3 : 1 ratio thus obtained demonstrated that female digamety of the Honduras population is made possible by the strong female action of the sex gene in the W (X) chromosome which outweighs the male valency of its allele in the Y chromosome from the Mexican parent.

Gordon's experiments showed that the sex determining mechanisms in the natural populations of British Honduras and Mexico are fairly stable although the absence of heteromorphic sex chromosomes and the appearance of a few sexually reversed phenotypes in laboratory stocks indicates the possibility that the "superior" sex genes in the sex chromosomes can be overridden by the influence of many opposing autosomal genes as in the case of *Lebistes*.

That this may occur has been indeed demonstrated by Öktay (1959) who has obtained a domesticated strain of *Xiphophorus maculatus* with XX males and XX females where polygenic determination takes place. McIntyre (1961) reported the occurrence of spontaneous sex reversal of genetic males from inbred stocks of known ancestry. This appears to have been the result of the accumulation of the female determining genes in the autosomes and of the poorly canalized development which arises in inbred strains.

The origin of the two types of sex digamety in the platyfish from Mexico and from British Honduras seems, however, to be due to a mutation of the superior sex genes in the sex chromosomes and not to the selection of minor autosomal sex genes which has taken place in strains of *Lebistes*.

The connecting element between the *Lebistes* and the *Xiphophorus maculatus* systems of sex determination seem to lay in the close similarity existing between X and Y chromosomes and in their scarce differentiation from autosomes. Both in the selection experiments in *Lebistes* and in the exceptional strains of *Xiphophorus maculatus* autosomal influences appear evident.

Kosswig's experiments showed that such differentiation is hardly possible among other species of the genus *Xiphophorus* and therefore the mechanism of sex digamety, which can be seriously compromised by the selection of autosomal sex genes in some *Lebistes* strains, is replaced by a mechanism of sex determination where either sex can produce gametes exerting widely varying influence in the sex determination of the progeny.

3. The Polyfactorial Sex Determination of Xiphophorus helleri

Xiphophorus helleri, *Platypoecilus variatus*, *Platypoecilus maculatus* and *Platypoecilus xiphidium* have been employed by Kosswig and co-workers in most of their investigations on sex determination of cyprinodont fishes. The three species of *Platypoecilus* are now generally included in the genus *Xipho-*

phorus but they differ from *helleri*, the earlier well known representative of the genus, in that they show a certain degree of sex stability after they have reached the age of reproduction. Some individuals of *Xiphophorus helleri* revert on the contrary from the female to the male sex (Essemberg, 1923, 1926) in spite of a marked sex dimorphism (Fig. 6.2) and many of the males of *helleri* are known to undergo a transient immature female stage (Friess, 1933; Regnier, 1938). *Xiphophorus helleri* has therefore been considered as a latent hermaphrodite animal (Kosswig, 1935).

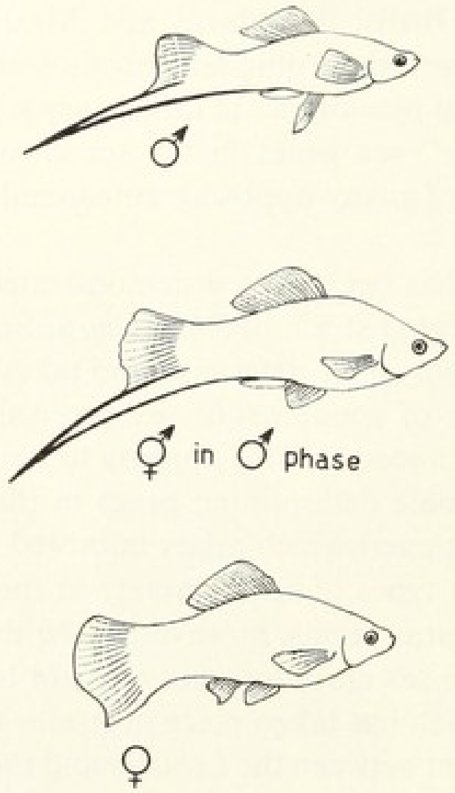

FIG. 6.2. Male, female and hermaphrodite individuals of *Xiphophorus helleri*. The hermaphroditic individual is in male phase and it shows its origin from a female phase individual being bigger than average "primary" males.

The work by Kosswig and his collaborators was based upon the use of interspecific crosses in establishing the localization of the sex determining genes and the idea of evaluating the efficiencies of the sex determiners on the basis of their influence in shifting sex ratios.

Kosswig's interpretations have been gradually extended and integrated and they were summarized in 1955 (Kosswig and Öktay).

The early work by Kosswig (1930-1933) showed a difference between the type of determination of *Xiphophorus helleri* and the type of determination of the three other species and a series of crosses established first of all what was called the homogamety of both male and female *Xiphophorus helleri* in relation to sex chromosomes. This amounts to saying that no sex chromosomes exist in *helleri* because the very mechanism and definition of such chromosomes (see p. 69) are based upon the digamety of one of the sexes, and that sex is determined by autosomal genes.

The hypothesis of the polyfactorial sex determination of *helleri* was therefore advanced by Kosswig as early as 1930 and it found strong support in the work of Breider (1935a) who demonstrated that *helleri* has male and female individuals of different "strength". The degree of strength of a male was evaluated upon the percentage of his male progeny with a given female and, on the other hand, the relative strength of different females was evaluated by crossing them with a given male. The comparison between the strengths of different males was made possible by crossing a single female with males that were marked with different genes for coloration. It was established in this way that a strong male produces more male progeny with a given female than a weaker male with the same female.

The sex of an individual is decided by the prevalence of the independent polymeric genes for one sex upon the sum of the genes for the opposite sex. Strong and weak sexes are thus due to the more or less strong heterozygosy of such polymeric sex factors. Such explanation, which has been reported from a recent work by Kosswig (1959), is not basically different from earlier explanations by Kosswig himself who assumed the existence of sex realizators, and therefore of the so-called AG system.

The sex ratio of the single families must be distinguished in this and in similar instances of polyfactorial sex determination from the sex ratio in a population since Friess (1933) obtained a 1 : 1 sex ratio from a number of crosses each of which gave widely different sex ratios in the progeny.

An interpretation similar to that proposed for *Xiphophorus helleri* holds true also for species of cyprinodonts of the genus *Limia* (Breider, 1935b).

Crosses between different species of *Xiphophorus* gave also interesting results some of which are summarized here.

Crosses of *helleri* ♀ × *maculatus* ♂ produced male and female progeny, a result which apparently contrasted with the results obtained in *Bryonia* (see p. 68) because the male sex is homogametic in *Xiphophorus maculatus*, and both sexes of *helleri* are homogametic according to the results of crosses between reversed sex phenotypes. Kosswig explained this result by assuming that the polygenic sex determination of *helleri* is epistatic upon the female digametic type of sex determination of *maculatus*.

The reciprocal crosses *maculatus* ♀ × *helleri* ♂ produced also male and female hybrids, and this result is in accordance both with Kosswig's explanation and with the assumption that the presence of male and female individuals in the progeny is due to the female digamety of the *maculatus* strain employed in the crosses.

Back-crosses between *helleri-maculatus* hybrids and *helleri* supported the former explanation because they gave progenies with sex ratios that varied according to the strengths of the *helleri* individuals introduced in the crosses. This result has been confirmed by Kosswig in thousands of crosses.

The almost complete absence of females in the progeny of the backcross between *helleri-maculatus* females with *maculatus* homozygote males (Fig. 6.3) seems to be due on the other hand to the segregation of the autosomal female sex genes of *helleri*. The normal sex ratios obtained from backcrosses with heterozygotic *maculatus* females has received the following explanation: the males arise through segregation of the autosomal F genes of *helleri* and the appearance of the females is due to the X of *maculatus* (Kosswig and Öktay, 1955).

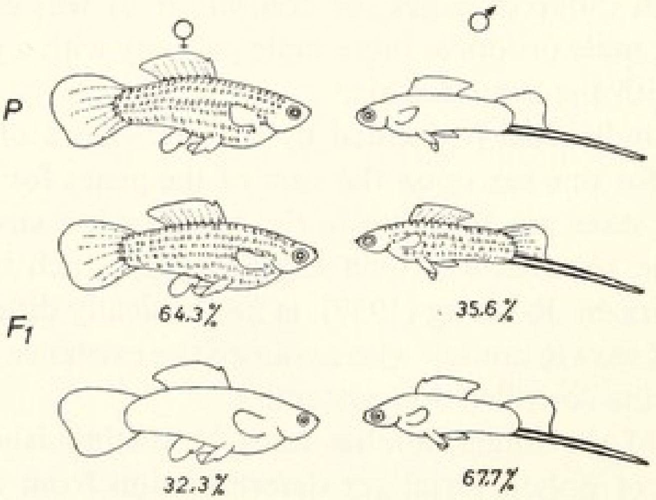

FIG. 6.3. Segregation in a cross of *Xiphophorus helleri*. The sex ratio is different within the two colour classes although the segregation for the colour gene gives a good 1 : 1 ratio (Kosswig, 1939a).

The male heterogamety of *Platypoecilus* (or *Xiphophorus*) *xiphidium* and *variatus* was established by Kosswig in 1935 and crosses of these species with *helleri* lead to similar conclusions regarding the polyfactorial sex determination of *Xiphophorus helleri* (Kosswig, 1939; Rust, 1941).

Kosswig attached also much importance in his earlier papers (1933) to colour genes which are localized in the sex chromosomes of the digametic species or in the peculiar autosome of *helleri* which can be regarded as homologous to the sex chromosomes of the other *Xiphophorus* species. They were considered as relative sex realizators on the basis of experiments which indicated some influences of colour genes upon sex ratios. Figure 6.4 shows that in *Xiphophorus helleri* the gene *Mo* (for *montezumae*) segregates regularly but when the dominant allele is present the percentage of females is higher than in the genotypes that are homozygous for the recessive allele *mo*. Figure 6.5 also seems to indicate a specific influence of genes for coloration in crosses between *helleri* ♀ × *maculatus* ♂. Although the idea of relative sex realizators was later momentarily abandoned by Kosswig and Öktay (1955), it has been recently supported by work on Crustaceans.

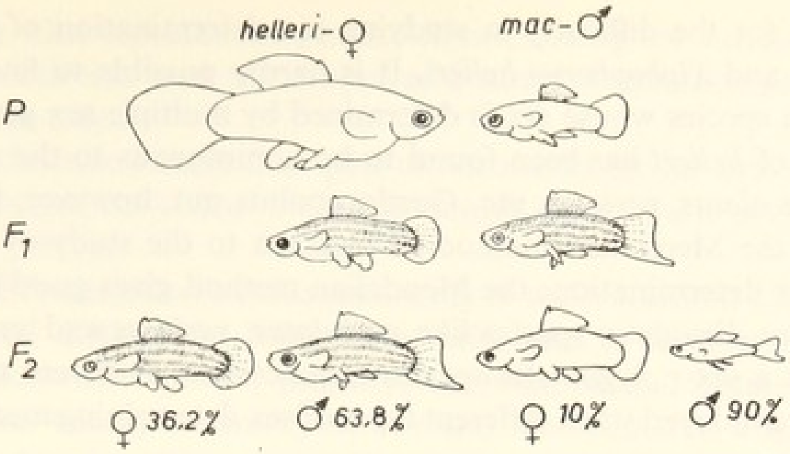

FIG. 6.4. The two classes of colour alleles in the F$_2$ generation in cross of *Xyphophorus helleri* ♀ × *X. maculatus* ♂ show peculiar sex ratios (Kosswig, 1939a).

The X chromosomes of *maculatus* and their homologous *x* chromosome in *helleri* were thought at first to be empty of the sex genes but Gordon's researches demonstrated later (1947) that the X chromosomes of *maculatus* from different sources have different sex potencies (see p. 120). When Kosswig and Öktay confirmed such experiments, they concluded that Z and X of *maculatus* and *variatus* have simply M and F genes of different strength and this accounts for the different sex ratios in the different colour classes. A close relationship between colour genes and sex genes and even an identity of such genes (conceived as functional units) cannot, however, be excluded, as suggested by Winge for the *maculatus* gene in the differential segment of Y in *Lebistes*.

It must be recognized that Kosswig's interpretation, without the support of additional hypotheses, leaves a few results still unexplained (Kosswig and Öktay, 1955) but the bulk of the data obtained from the study of *Xiphophorus helleri* and its various hybrids is satisfactorily explained on the basis of Kosswig's theory.

The lack of sex-linked characters was considered by Gordon (1957) as the

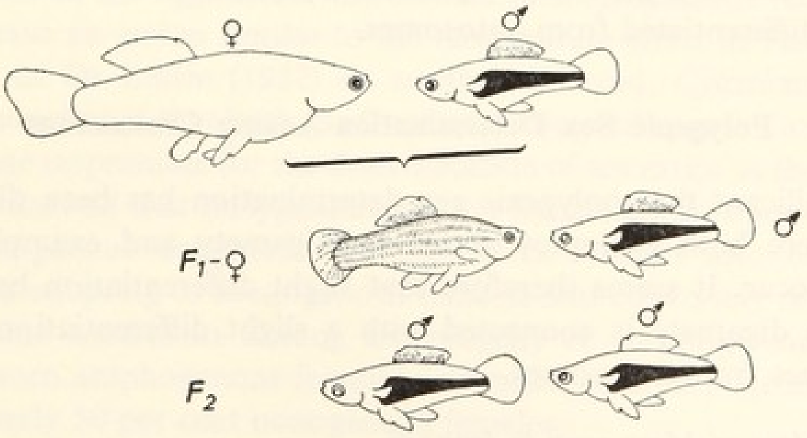

FIG. 6.5. Sex ratios and colour in a back-cross between a female hybrid *maculatus* × *helleri* and a male *maculatus* (Kosswig, 1939a).

chief reason for the difficulty in studying sex determination of *Xiphophorus montezumae* and *Xiphophorus helleri*. It is hardly possible to find sex-linked characters in species whose sex is determined by multiple sex genes even if a *x* autosome of *helleri* has been found to be homologous to the sex chromosomes of *maculatus*, *variatus*, etc. Gordon points out, however, the difficulty of applying the Mendelian method of research to the study of a system of polygenic sex determination: the Mendelian method gives good results when applied to sex digametic species like *maculatus*, *variatus* and *xyphidium* but it fails when a sex polygametic species is concerned. Different criteria must therefore be employed when different types of sex determining mechanisms are concerned.

Hämmerling (1937, 1947) tried to give an alternative explanation of the results by Kosswig by assuming that *helleri*'s plasma has feminizing properties which are absent in the *maculatus* cytoplasm. This interpretation appears, however, inconsistent with many of Kosswig's results and it has lost much of its value after Goldschmidt's hypothesis of the female determining action of the cytoplasm in *Lymantria* was abandoned. Hartmann has also advanced the idea (1955) that the sex ratios in *Xiphophorus* are actually altered by the cytoplasmic influence and by sex modifiers but that no true sex genes are involved in the system of polygenic determination. Goldschmidt advanced similar alternative explanations of the results (1937) and eventually accepted (1955) Hartmann's conclusions in a discussion of the De Lattin's work on isopods that lead to results similar to those obtained by Kosswig in ciprinodonts.

The existence of sex modifiers has repeatedly been assumed as an alternative to the action of true sex genes whenever new results have put into discussion the possibility that the simple mechanism of "monofactorial" sex determination could operate in some gonochorist species.

As a matter of fact a sharp distinction between sex genes and sex modifiers cannot easily be made even in species like *Drosophila*, where sex digamety is well established. It is generally impossible in species where sex chromosomes cannot be differentiated from autosomes.

Polygenic Sex Determination Among Crustaceans

It is significant that polygenic sex determination has been discovered in groups where both examples of female digamety and examples of male digamety occur. It seems therefore that slight differentiation between male and female digamety is connected with a slight differentiation of the sex chromosomes from autosomes.

1. The Problem of Monogeny in Isopods

A tendency to hermaphroditism, which has been noticed in *Xiphophorus* *helleri* and in other ciprinodonts, is also shown among the oniscoids, a group of isopods which has been the subject of a series of excellent papers on the problem of sex ratio.

Arcangeli described some examples of hermaphroditism among oniscoids (1932) and pointed out to the existence of strong variations in sex proportions among them (1931).

Vandel (1938) carried on a systematic study of such variations in *Trichoniscus provisorius* and distinguished two main categories of females: the amphogenous females with 1 ♂ : 1 ♀ progeny; the monogenous females having an offspring either wholly or mostly of one sex. He defined also six groups of monogenous females: (1) completely arrhenogenous females, that produce only male progeny; (2) incompletely arrhenogenous females, producing also a few daughters; (3) completely thelygenous females, producing only female progeny; (4) incompletely thelygenous females that produce only a few males; (5) allelogenous females which produce only males or only females in different batches; (6) mixed females, that are amphogenous, but are born from monogenous mothers.

Vandel's observations demonstrated that amphogenous females crossed with males of amphogenous origin produce half male and female progeny. Females of thelygenous origin produce only monogenous daughters. Thelygenous females produce either only thelygenous females or arrhenogenous females or both. Thelygenous or mixed females may occasionally be produced by exceptional females, daughters of an arrhenogenous mother which, however, generally originate arrhenogenous daughters. Both arrhenogenous and thelygenous daughters and sometimes only arrhenogenous daughters are produced by allelogenous females. The so-called mixed females produce arrhenogenous or incomplete thelygenous daughters or daughters of both types.

Any influence of the male parent must be excluded according to Vandel in the process of monogeny. Females are digametic and directed meiotic divisions occur in the eggs under the control of a cytoplasmic factor, which appears to have an action similar to the temperature effect in *Talaeporia*.

The work of De Lattin (1952) on another oniscoid, *Cylisticus convexus*, actually demonstrated that both the female and the male parent, and not only the female, are responsible for the determination of sex ratios in the offspring.

De Lattin showed that thelygenous females frequently produce one half or more arrhenogenous daughters. Amphogenous females also occasionally appear in the offspring of monogenous females and conversely monogenous females appear sometimes among the progeny of amphogenous mothers. Crosses between amphogenous females and males of arrhenogenous descent produced nearly 50 per cent nonogenous females.

A marked influence of colour genes on sex ratios was demonstrated by De Lattin in other species (1951) of oniscoids and also in *Cylisticus convexus*

(1958) in accordance with the previous results by Kosswig on the influence of colour genes in the sex ratios of *Xiphophorus* species.

De Lattin assumed accordingly that F and M realizator complexes are distributed along autosomes. Among the realizators of the F complex a dominant F' is assumed to have a predetermining action and to exert its influence from the mother both in F' and in f'f' individuals. Another gene, I, is supposed to increase the feminizing action F' and therefore genotypes carrying F' and I determine feminization, as F' is epistatic over all other sex realizators in the presence of I. Additional hypotheses were advanced in order to explain all the results of crosses in *Cylisticus* and they seem to have stimulated Goldschmidt's strong criticism (1955) of the monstrosity of polyfactorial theory of sex determination.

And yet the main assumption by De Lattin that both male and female parents take part in the determination of monogeny is fully demonstrated by the results of the crosses and the strong influence of colour genes is evident. Goldschmidt assumed that the cytoplasmic control mechanism is susceptible to genetic or environmental modifications, which is only a different way of saying that monogeny is actually determined by the environment or by genetic modifiers.

Legrand described rudimentary hermaphroditism (1947) and the occurrence of female intersexes (1956) in *Porcellio dilatatus* and advanced the idea that major and minor genes are present in the sex genotype, the latter being able to cause a certain degree of intersexuality when the F and M complexes are in peculiar conditions of equilibrium.

A brilliant confirmation of the idea of multifactorial sex determination in oniscoids has been provided by Johnson (1961) who employed in his crosses distant populations of *Haplophtalmus danicus* and was thus able to utilize a certain amount of sex variability of geographic origin. Sixteen thelygenous and nine amphogenous strains of the species were collected at Poitiers, seventy-nine amphogenous, four arrhenogenous and no thelygenous strains were collected at Fouras in France. The strains from Fouras showed stable amphogeny. Only one among the nine amphogenous strains from Poitiers was stable and the thelygenous strain resulted partly stable and partly unstable.

Crosses of females from stable thelygenous strains with arrhenogenous males from Fouras have thelygenous females. The F_2 and back-crosses showed arrhenogenous, amphogenous and thelygenous offspring. Crosses of stable thelygenous females with amphogenous males from Fouras gave similar results with an higher percentage of arrhenogenous offspring in the F_2 generation. Crosses between both stable and unstable thelygenous females with males from different localities gave in general a high percentage of male progeny both in F_2 and as a result of back-crosses.

Thus crosses between what can be called different sexual races of *Haplo-*

phtalmus have basically confirmed De Lattin's results and interpretations. The action of F and M gene complexes can be modified by minor genes and by a cytoplasmic factor in three different sexual categories: (1) the stable amphogenous complex where the action of major sex genes is inhibited by modifying genes; (2) the stable thelygenous complex where the epistasy of F genes is obtained both by means of modifiers and by a X cytoplasmic factor; (3) the genic complex of the mixed group, including unstable amphogenous and thelygenous strains, originates progenies of thelygenous, amphogenous or arrhenogenous type through the action of modifiers that enhance either the action of the F or of the M complex or both in the absence of the X factor.

The influence of the males of different origin in changing the percentage of the males in the progeny is explained through the assumption, which is based on a number of similar well-known examples, that the major F genes may change from a dominant to a recessive condition when introduced in a different genotype. This effect would be increased by the cytoplasmic X factors in the presence of a genic complex other than that of Poitier.

The problems of monogeny are thus approaching a satisfactory solution on the basis of the so-called multifactorial interpretation thanks to the gradual introduction of methods and concepts of population genetics. Howard's (1942, 1958) and Patanè's (1955) crosses in other oniscoids appear to be susceptible of similar interpretations despite the comparatively limited number of the individuals examined.

The early researches by Vitagliano Tadini (1958) and by Montalenti (1960) on *Asellus aquaticus*, a freshwater isopod, seemed to support the hypothesis of poligenic sex determination, which has, however, been subsequently abandoned on the basis of new results in *Asellus*. According to the authors (Montalenti and Vitagliano, 1963) a limited number of closely linked genes or gene blocks would be involved in the sex determining mechanism of the *Asellus aquaticus* population from the River Sarno near Naples. Males would belong to three genotypes with two, three or four dominants and females would belong to two genotypes with one or no dominants.

The hypothesis is very appealing in its simplicity but experimental data have not been published yet. An oligofactorial type of sex determination would represent an intermediate step between polyfactorial and monofactorial types of sex determination.

An oligofactorial mechanism of sex determination appears, however, inadequate for the interpretation of many significant facts that have been discovered in relation to monogeny in species of oniscoids. The relationship between colour genes and sex genes, between nuclear and cytoplasmic mechanisms of sex determination can only be guessed at the present confused stage of our knowledge. It is only certain that some populations of isopods present multiple sex genotypes in the absence of much phenotypic variability.

2. Environmental Factors, Colour Genes and Sex in Amphipods

The study of genetic and environmental factors controlling sex in amphipods has begun in recent years but it has already shown the existence of some interesting mechanisms.

The sex ratio of the brackish water amphipod *Gammarus salinus* is shifted, according to Kinne (1952, 1953), in favour of the male sex at low temperature conditions (5°C) and in favour of the female sex at abnormally high temperatures (22° and 23°C) and the critical period for the influence of the temperature conditions corresponds to the last phase of eggs maturation. This result has been taken by the author as a demonstration of a primary control by the temperature conditions on sex determination but the later experiments by Metzler on *Cyclops viridis* (see p. 85) suggest the possibility of a differential influence on the sex chromosome in a case of female digamety. It is true that Niiyama has demonstrated the existence of XY chromosomes in the male sex of *Anisogammarus anandalei* (1950) but clear examples of polyfactorial sex determination in other gammarids render quite plausible the existence of both male and female digamety in the family. It seems therefore that the question of sex determination in *Gammarus* requires a careful cytological control.

The problem of polyfactorial sex determination and of the relationships between colour genes and sex ratio in amphipods has first been approached by Anders (1957) in *Gammarus pulex subterraneus*. A series of autosomal multiple alleles (R^2, r, r) is responsible in this species for a brown, olive and red coloration respectively. The normal allele r^+ does not influence the normal sex ratio, the R^2 allele has a masculinizing effect and a yet stronger effect is exerted by the r allele.

The problem of monogeny in amphipods has recently been studied by Traut (1961) in strains of *Gammarus duebueni* from the lower Elbe and from Kiel. Both populations were composed of thelygenous, amphogenous and arrhenogenous females. Allelogenous pairs were also obtained.

Traut reached the conclusion that sex is determined in amphogenous, arrhenogenous and amphogenous strains through the work of a polyfactorial system of sex realizators which is also influenced by sex modificators. Thelygeny seems, on the contrary, to be determined by cytoplasmic influences. In other words sex is determined by autosomal genes and by cytoplasmic factors as in some oniscoids. An influence of temperature conditions has been ruled out in the case of *Gammarus duebueni* and of *G. chevreuxi*.

The sex of the last species seems to be determined by a normal mechanism of homo-heterogamety.

Sex determination of gammarids appears therefore to be determined by mechanisms that are oriented in some instances toward pure sex digamety and show in other instances a puzzling variety of determining factors.

3. *Polygenic Sex Determination in the Marine Copepod* Tisbe reticulata

The genetics of *Tisbe reticulata* was first studied by Bocquet (1951) on populations of the coast of Bretagne and Battaglia has subsequently made extensive researches (1958–1961) on strains from populations living in the lagoon of Venice, where special adaptations to life in brackish waters have taken place. Differences in the genetics of the Atlantic and Mediterranean populations have been established by Battaglia who has especially studied the closely related problems of sex determination and of balanced polymorphism.

The starting point of Battaglia's work on sex determination was the realization that the percentage of females is higher among the heterozygotes for *violacea maculata* than in the homozygotes for either colour gene. The colour patterns in the population of the lagoon of Venice appear to be due to a series of at least three alleles, two of which are dominant over a common recessive. The phenotype *violacea maculata* is a heterozygote for the V^v and V^m alleles, which—according to Battaglia—may also belong to closely linked loci.

Selection experiments in *Tisbe reticulata* and in the related species *Tisbe gracilis* showed on the other hand that inbreeding leads to the production of 100 per cent males within five to six generations and that a sudden increase of the percentage of the females is obtained when inbreeding is broken. It appears thus that the high percentage of females in the *violacea maculata* phenotype is the expression of a certain degree of heterozygosis rather than the expression of the influence of colour genes on sex. In other words femaleness is favoured by heterozygosis and maleness by homozygosis, a mechanism which closely resembles the mechanism of sex determination analysed by Whiting and co-workers in *Habrobracon* (see Chapter 10).

Battaglia assumes in his interpretation of the experiments the existence of several dominant factors for femaleness and of multiple recessive factors for maleness which are located in different loci of different chromosomes. The F factors are expressed, or their expressivity is increased, in heterozygous condition and, on the contrary, the recessive *m* alleles can only be expressed in a homozygous condition. The example of the heterozygotes for *violacea maculata*, where females are in higher percentage than in the two corresponding homozygotes, gives a visual demonstration of this interpretation which fits with all the facts established so far in the sex determination of *Tisbe*.

An heterozygous sex is thus obtained in a sex determining system that may be regarded as polygenic and it appears that such system is maintained through delicate mechanisms of balanced polymorphism which have been partially investigated by Battaglia (1958, 1961). The heterozygosis of one sex is maintained in the advanced systems of sex determination by the more crude mechanism of the sex chromosomes.

The mechanisms of polyfactorial sex determination among crustaceans appear therefore to be rather varied. While in the case of the oniscoids the M/F balance appears to be the result of a balance between M and F genes and modifiers, in the case of *Tisbe* it results from the homozygosis of the recessive *m* factors and the heterozygosis of the F factors.

A common feature of the different types of polyfactorial sex determination consists, as Kosswig pointed out, in an extremely precise working of the switch mechanism which, in the absence of sex chromosomes allows the appearance of normal male and female phenotypes even in the cases of a nearly perfect equilibrium between male and female determiners.

The Progamic Sex Determination of *Dinophilus apatris*

Dinophilus apatris is cited as the typical example of the so-called progamic sex determination, that is of a kind of sex determination which takes place before fertilization. Hartmann (1943) included *Dinophilus* in the heterogeneous category of the organisms showing phenotypical determination of sex upon the assumption that the sexualization of the two kind of eggs of *Dinophilus* is determined by environmental factors of unknown nature.

Korschelt discovered in 1882 that the female of *Dinophilus* lays within the same capsule a few big and a few much smaller eggs (Fig. 7.10): the bigger eggs produce female and the smaller eggs produce male individuals. De Beauchamp demonstrated in 1910 that unfertilized females are able to lay larger sized and smaller sized eggs from which females and males originate respectively. This observation rules out the possibility of a determination of the sex during the formation of the zygote from which each individual is developed (the so-called syngamic sex determination).

Researches by Malsen (1906) and by Tsonis (1939) demonstrated that temperature, quantity of food and the addition of certain salts to the water alter the sex ratios between the two types of eggs which, following Shearer (1911) and Nachtsheim (1920), can be named male and female eggs.

Such environmental influences, however, can only partially explain the wide differences in the sex ratios of the two types of eggs which range between 1 : 1 (Prowazek, 1900) and 1 : 2·5 (Shearer, 1911) in different strains.

Nachtsheim (1920) also observed the existence of different sexual races of *Dinophilus* where the sex ratios are constantly different under the same environmental conditions. Such results were confirmed in 1956 by Bacci who found a sex ratio of 1 : 0·6 in strains of *Dinophilus* from Roscoff on the Atlantic coast, 1 : 1 in strains from Margellina in the Bay of Naples and 1 : 2 in strains from Fusaro, a slightly brackish lake near Naples. The cultures of three strains were made in water from the Bay of Naples using *Chlamydomonas* as food.

The hypothesis of some kind of sex determination based on the sex digamety of one sex can be ruled out both on the basis of the abnormal sex proportions and on the basis of Nachtsheim's cytological work which showed that sex is determined before meiosis takes place in the germinal cells of the female. A totally phenotypical type of sex determination in the sense of Hartmann (see p. 137) can likewise be excluded on the ground of the constant racial differences of the sex ratios observed in uniform environmental conditions.

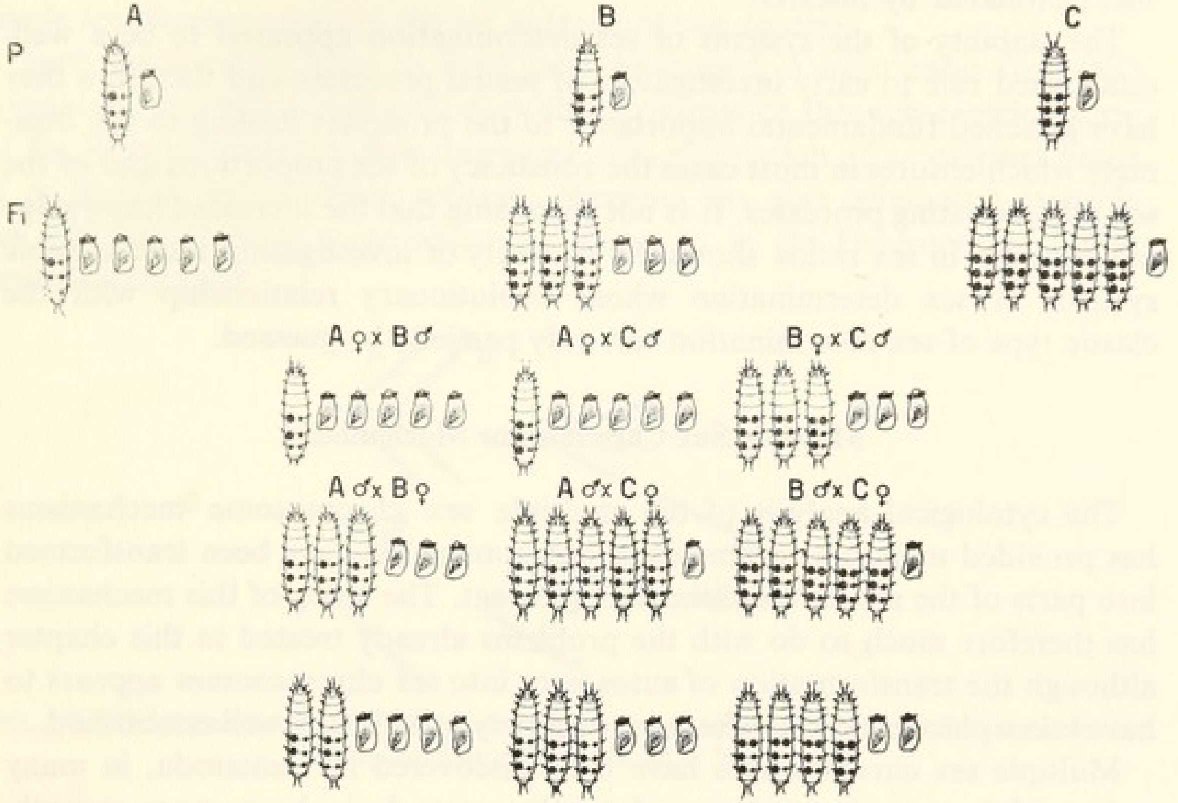

FIG. 6.6. An interpretation of the different sex ratios in different strains of *Dinophilus apatris* (Bacci, 1956).

The following hypothesis, illustrated in Fig. 6.6, has therefore been made to interpret sex determination in the well-known case of *Dinophilus* (Bacci, 1956). The formation of the two types of eggs is under the influence of the maternal sex genotype and the ratio between male and female eggs is the result of the balance between multiple F and M factors in the genotype of the mother. As a result of meiosis and of fertilization the resulting zygotes will have sex genotypes which will be either similar or very different from the maternal genotype. And female phenotypes, differentiated as such under the influence of the maternal genotype, may actually have a prevalent male genotype which, however, will find expression in the prevalent production of male eggs and thus in the higher frequency of males in the offspring.

This interpretation can be visualized in Fig. 6.6 where a strain with a prevalence of male eggs, a strain with a prevalence of female eggs and a strain with a 1 : 1 ratio are represented with the result of their crosses under the assumption of a polyfactorial sex determination and of a maternal effect.

Goldschmidt advanced in 1955 the hypothesis that the female might be a XXY with a strong female determiner in the Y and that in oogenesis the Y might be removed in future male eggs but the cytological research by Nachtsheim had already excluded the occurrence of a similar phenomenon which was postulated by Shearer.

The stability of the systems of sex determination appeared to be a well-established rule to early investigators of sexual processes and therefore they have attached fundamental importance to the processes leading to sex digamety which ensures in most cases the constancy of sex proportions and of the sex differentiating processes. It is not surprising that the increased knowledge of variations in sex ratios shows the necessity of investigating more suitable systems of sex determination whose evolutionary relationship with the classic type of sex determination can only partially be guessed.

Multiple Sex Chromosome Mechanisms

The cytological analysis of the multiple sex chromosome mechanisms has provided numerous examples of autosomes that have been transformed into parts of the sex chromosome complement. The study of this mechanism has therefore much to do with the problems already treated in this chapter although the transformation of autosomes into sex chromosomes appears to have taken place in species where sex digamety was already well established.

Multiple sex chromosomes have been discovered in Nematoda, in many orders of Insecta, in spiders and similar cases have been more recently described also in Ostracoda (Bauer, 1940; Dietz, 1958) and in isopods (Steiger and Bocquet, 1954).

Most important of all, from the point of view of sex determination, seem to be the multiple sex chromosome mechanisms which are concerned with the more or less regular appearance of males in heterogonic species but they will be dealt with in a following chapter. Some groups (like Mantoidea and Orthopteroidea) provided very good material for a detailed cytological analysis and, although experimental work is lacking in this field, cytologists were able to provide fine pictures of the evolution of sex chromosomes in such instances.

Before advancing into this field it is nevertheless convenient to illustrate the classical example of the beetle *Blaps lusitanica* as described by Nonidez (1920). A sex quinquevalent is present during spermatogenesis at the first meiotic division and the Y regularly segregate from the X's at the anaphase of the first meiotic division. Two kind of sperms are thus formed and the male has $X_1X_2X_3X_4Y$ sex chromosomes although the possibility of the existence of multiple Y chromosomes has not been ruled out. A re-investigation of the cytology of the genus *Blaps* (Guenin, 1948, 1950) substantially confirmed the previous results and led to the discovery of a species *Blaps polycresta* from Palestine whose caryotype is:

$$2\,n\,\male = 18 + 12\,X + 6\,Y = 36$$

$$2\,n\,\female = 18 + 24\,X = 42.$$

It would be extremely interesting to be able to evaluate the influence of the single sex chromosomes in the sex balance of this species.

Such mechanisms seem to have been developed in most cases from XO conditions through translocations with autosomes or through centric fusion of an X chromosome and an autosome. Fragmentation of an original single X or Y into two or more separate elements has also originated multiple sex chromosomes in some instances where, as in the Heteroptera, diffuse centromeres exist.

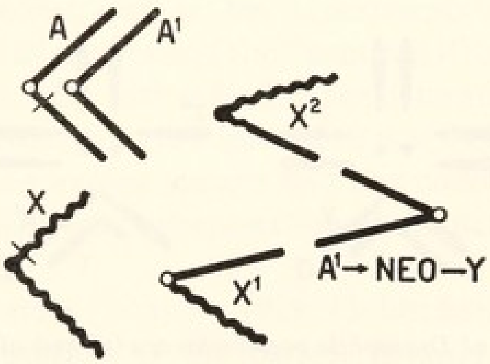

FIG. 6.7. The evolution of an X^1X^2Y mechanism from an original XO type of male digamety (Hughes Schrader, 1950 after White).

A diagrammatic representation of the origin of an X^1X^2Y mechanism from an original XO type male has been outlined by Hughes Schrader (1950) after White's (1941) results on sex chromosome mechanisms in some species of mantids. In Fig. 6.7 solid outline indicates the euchromatin of autosomes, wavy outlines the heterochromatin of the original X chromosome. Fine lines mark the position of breaks. The females of a number of sub-families of Mantoidea are $X_1X_1X_2X_2$ and it is very probable that all these sub-families have originated from a single species so that neo-X_1X_2Y derivation from an XO condition has very likely evolved along a single line. Considerable changes in form and in degree of heterochromatinization of the Y chromosome, which is confined to males, have taken place inside the different genera as the only limits to its variation are given by the retention of sufficient homology for pairing with both X elements and by the retention of genetic requirements for viability and fertility. The length of the neo-Y—as compared to the arms of the two X's with which it pairs—is most reduced in the genus *Melliera* and it shows no reduction in the two genera *Choeradodis* and *Stagmantoptera*. Some other Orthopteroid species have evolved similar mechanisms although the mantid case is up to now the best analysed in this field.

The sex chromosome mechanism of *Drosophila miranda*, which is closely related to *D. pseudoobscura* and *D. persimilis*, has developed from the XY: XX mechanism which is present in related forms. The male of *D. miranda* has nine chromosomes instead of ten in the diploid set and the missing element is one member of the third pairs of autosomes. This chromosome, which is acrocentric, has become a sex chromosome and always passes to the same pole as X in the male. The metacentric X, which is homologous to the X chromosome of *D. pseudoobscura* and of *D. persimilis*, is designated as X_1, the new sex chromosome is referred to as X_2. A sex trivalent is formed during spermatogenesis (Cooper, 1946) and X_1 pairs with one part of the Y chromosome, X_2 with another part which is probably derived from the missing third chromosome, X_1 and X_2 segregate therefore to the opposite pole at anaphase (Fig. 6.8).

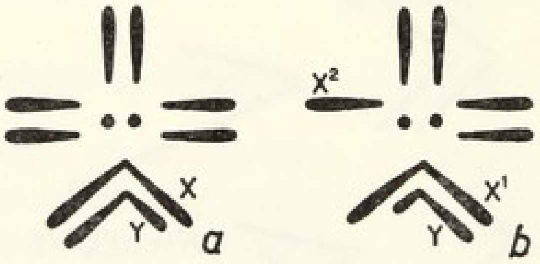

FIG. 6.8. Male caryotypes of *Drosophila pseudoobscura* (a) and of *D. miranda*: a member of the third pair of chromosomes has become an heterochromosome (X^2) in *D. miranda* and is homologous to part of the Y chromosome (based on various authorities).

In *Drosophila americana americana* a centric fusion has taken place between the X and one member of the fourth pair of autosomes. The other member of the fourth pair has thus become restricted to the male line and can be considered as a Y_2 (Stone and Patterson, 1947).

Many more cases of multiple sex chromosome mechanisms have been accurately investigated as their analysis provides an excellent basis for studies in cytotaxonomy.

The major contribution to the knowledge of the actual sex determining mechanism in cases of multiple sex chromosomes is provided by the work of Beerman on *Chironomus* (1955). Two types of males, $X_1Y_1X_2X_2AA$ and $X_1X_1X_2Y_2AA$, are present in the same population with only one category of females, $X_1X_1X_2X_2AA$. Although the localization of the female determining genes could not be established, it was ascertained that a male determining section is attached to a Y chromosome.

The major significance of the study of multiple sex chromosomes in the problem of sex determination consists at present in the fact that they provide visible examples of the integration of autosomal elements in the system of sex chromosomes.

CHAPTER 7

SEX PHENOTYPES IN HERMAPHRODITISM AND IN RELATED SEX CONDITIONS

IT has been a general opinion both among botanists and zoologists that hermaphrodite species exhibit a single sex phenotype where male and female gametes mature at the same time or in successive phases of the individual development.

Correns (1907) demonstrated in his experiments on *Bryonia* both the digamety of the dioecious species and the "neutrality" of all the gametes from the monoecious *Bryonia alba* with regard to sex determination in the hybrids. Thus the *Bryonia* experiments were regarded as proof of the uniformity of the sex genotype in the monoecious species and this conclusion was considered of general value for a genetical interpretation of sex determination in hermaphrodites.

It was assumed therefore (Witschi, 1929; Goldschmidt, 1931) that hermaphroditism is the expression of the perfect balance of male and female factors (F and M) in each individual and the general formula FFMM, where $F = M$, was accepted for diploid hermaphrodite species. All the gametes would thus be perfectly uniform with regard to sex-determining genes in a condition of what may be called sexual monogamety as opposed to sex digamety.

Hartmann (1943) linked to hermaphrodite species also those unisexual species whose sex appeared to be determined exclusively by environmental factors and assumed that male and female individuals of such species have the same genetic constitution with regard to sex factors. The same view was supported by Hauenschild (1953) who concluded that in species showing the so-called phenotypical sex determination all gametes carry F and M factors of equal strength. Hartmann later replaced the term "phenotypical sex determination" with that of "modificative sex determination" (1956) but the concepts and formulations have remained unchanged.

The discovery of a high degree of sex variability in some hermaphrodite species has recently led to a new interpretation of sex determination in hermaphrodites. The study of the hereditary determination of any biological character requires in fact a precise and extended knowledge of its variability and any theory that is not based upon such knowledge is inadequate from a genetical point of view.

It is important therefore to make a preliminary survey of both the individual variability and of the variability of the sex phenotypes in the hermaphrodite populations and in the unisexual species whose sex phenotype is deeply modified by ecological environmental influences.

From False Gonochorism to Rudimentary Hermaphroditism

1. False Gonochorism in Coris julis

Coris julis is a fish of the labrid family which was considered to be closely allied to the species *Coris giofredi* that lives on the same shores in the Mediterranean. *Coris julis* is brightly coloured and bigger than *C. giofredi* (Fig. 7.1) and it was known that *julis* specimens are generally, but not always, male and *giofredi* specimens generally female. A statistical examination of a

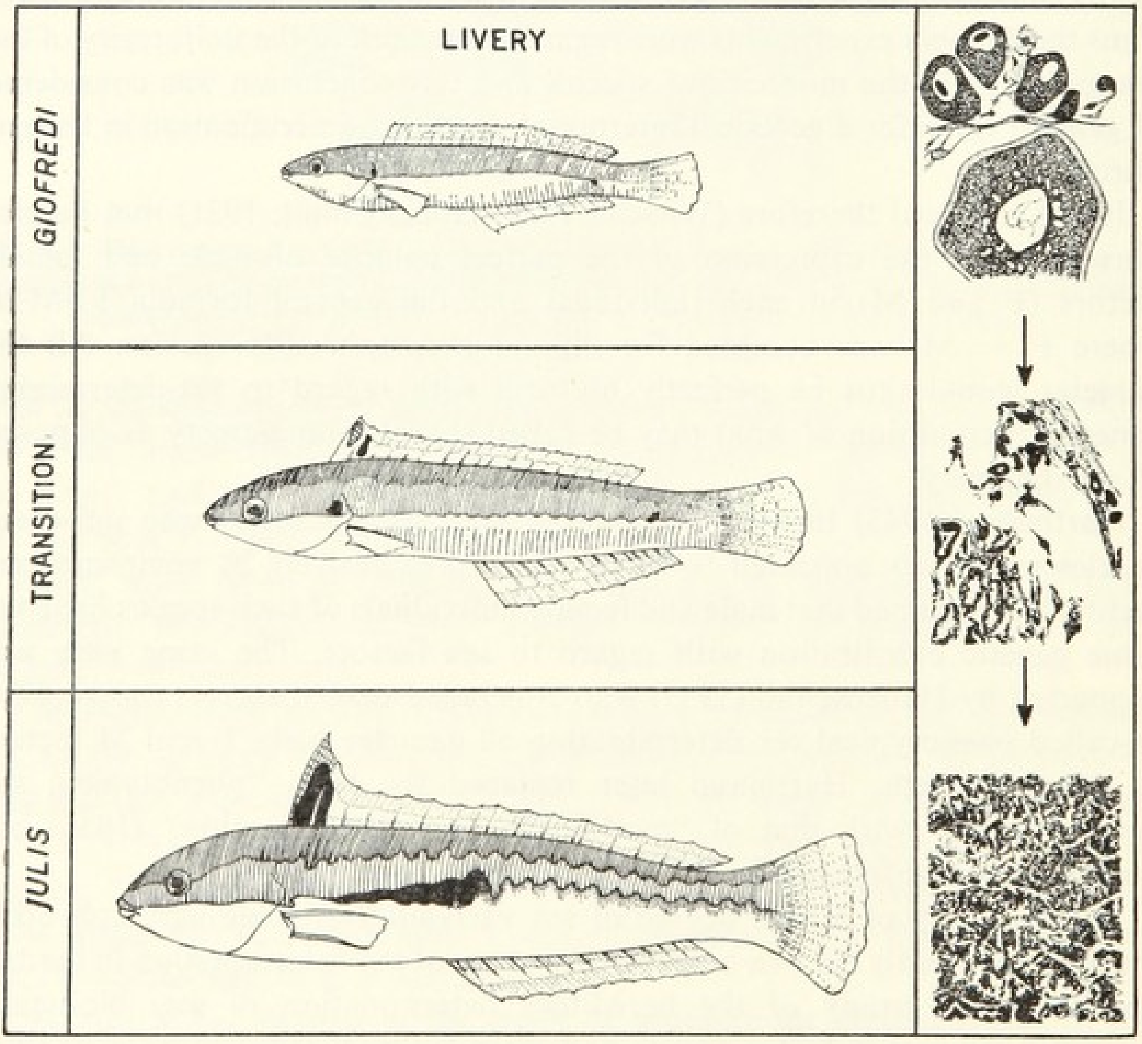

FIG. 7.1. Change of livery and change of the sex phase in *Coris julis* (after Bacci and Razzauti).

few hundred *Coris* demonstrated that the few male *giofredi* specimens belong to the bigger-size classes of this form and the female *julis* specimens belong on the contrary to the smaller size classes. Transitional conditions between the *giofredi* and the *julis* liveries were discovered and histological examination demonstrated the presence of oocytes in male gonads of a few individuals in the *giofredi*, in the transitional and in the *julis* liveries. Integration of statistical and cytological data allowed one to conclude (Bacci and Razzauti, 1957) for the existence of protogynous hermaphroditism in the species *Coris julis* which shows three different liveries (Fig. 7.1).

The widespread occurrence of protogynous hermaphroditism in species of the labrid family has been demonstrated by later researches (Sordi, 1962).

This is only one of the most striking examples of false gonochorism (Bacci, 1955) which is shown by animals that present during long periods of their life-cycles exclusively male or female gonads, and they resemble therefore unisexual animals, although at a certain stage they shed the sexual germinal elements of one sex which are rapidly replaced by elements of the other sex. False gonochorists may be regarded therefore as examples of consecutive hermaphroditism where the transitional hermaphrodite phase is very short (Bacci, 1951).

The existence of this kind of hermaphroditism in species that were previously considered unisexual can be demonstrated through observations in cultures, as in the case of bivalves and of cyprinodont fishes, or through integration of statistical and histological methods. The ever-growing number of instances of false gonochorism that are appearing in the zoological literature leads to the general conclusion that this kind of hermaphroditism is far more widespread among animals than it may appear in the present very limited stage of knowledge of animal groups from the point of view of sex conditions.

2. Sex Variability of Patella

The common limpet *Patella coerulea* from the Mediterranean is also a false gonochorist which undergoes transition from a male to a female phase after the end of the breeding season (Bacci, 1947b). The great number of individuals examined (about 40,000) in a population from the Bay of Naples allowed one to establish that there is in *Patella* a majority of hermaphrodite individuals which show equal duration of the male and female phases and that there is a small proportion of limpets where one of the two sexual phases is extremely reduced or abolished altogether. They are called pure males and females. Also among the protandrous individuals there is a strong variability: some of them have a shorter male, some a longer female phase and there is a series of intermediate sex phenotypes between pure males and females through the various degrees of protandrous hermaphrodite individuals.

This situation is clearly shown in Fig. 7.2 which indicates gradually decreasing proportions of male phase individuals with the increase in size and therefore with the age of the individuals. Histological investigations confirmed that passage from male to female phase occurs at any size class. The existence of a few female individuals in the smaller size classes of sexually differentiated individuals and of male phase specimens in the higher size classes shows on the other hand the existence of pure or almost pure male and female phenotypes.

Earlier statistical researches by Orton (1928), which were later integrated with cytological investigations by Bacci (1952), clearly demonstrated the existence of a similar situation in *Patella vulgata* from the English waters, although Dodd (1956) detected only examples of accidental or developmental hermaphroditism in the three British species of *Patella*.

Patella has been considered as the type of unbalanced hermaphroditism (Bacci, 1949a) which occurs in a number of bisexual populations showing a high variety of sex phenotypes.

The investigation of *Patella* allowed us to correlate and partially to re-interpret the results of an important series of researches on other molluscs like *Ostrea* (Orton, 1927), *Teredo* (Coe, 1943) and *Crepidula* (Orton, 1912; Coe, 1948), where the existence of purely male and female individuals and of hermaphrodites with different degrees of maleness and femaleness in natural populations is more or less clearly demonstrated.

The alternation of sexual phases in *Ostrea* and in *Teredo* and the influence of environmental factors in the growth of *Crepidula* (Gould, 1917, 1919) prevented us from having a clear idea of the continuous variation of sex phenotypes in these instances. An example of extreme variability in hermaphroditism among lamellibranchs is also shown in *Chlamys varia* according to research by Dalmon (1938) and by Lucas and Franc (1962).

Subsequent research and also a re-examination of former data showed that such instances of extreme variability of sex phenotypes in prevalently hermaphrodite populations are frequent not only among hermaphroditic molluscs but in other animal groups. Vannini (1960) has found an example of unbalanced hermaphroditism in *Hydra vulgaris*. Among polychaetes pure males and females are known in the hermaphrodite species *Platynereis dumerili* (Abeloos, 1950), *Pomatoceros triqueter* (Foyn, 1950) and *Hydroides norvegica* (Ranzoli, 1954, 1962).

Clear examples of unbalanced hermaphroditism have also been discovered by Durchon (1951, 1959) in the course of his work on stolonization in different species of syllids. Some individuals belonging to the species *Syllis amica*, *Syllis vittata*, *Syllis prolifera* and *Trypanosyllis zebra* change their sexual phase and some of them retain either the male or the female sex in the course of successive stolonization processes. Each species is composed therefore by false gonochorists, which seem to form the majority, and by less labile individuals that correspond to the pure males and females. Environmental factors play an important role in inducing sex reversal in these polychaete species as it was shown also by Hauenschild's researches (1953) on *Grubea clavata*, another syllid.

Pure males and females and protandric hermaphrodites were discovered (Arcangeli, 1923) in a Sardinian sub-species of the isopod *Philoscia elongata*.

Unbalanced hermaphroditism has been found among asteroids in the Mediterranean race of *Asterina gibbosa* (Cuenot, 1898; Bacci, 1951) and the examples of extreme sex variability discovered by D'Ancona (1951) in the hermaphrodite genera of teleosts *Pagellus* and *Diplodus* can probably be interpreted as cases of unbalanced hermaphroditism.

Marked differences in sex phenotypes have been observed in several hermaphrodite populations also in the absence of pure males and females.

3. *Uniformity of the Sex Phenotype in* Calyptraea

All the individuals of the gastropod *Calyptraea chinensis* from the Bay of Naples are protandrous hermaphrodites and each of them passes from the male to the female phase between the first and second year of age (Bacci, 1951). This extreme uniformity of the sex phenotypes is shown in Fig. 7.2 where the percentage of male phase individuals falls from 100 per cent in the class of 5 mm in length to 0 in the 10 mm class. No female phase individuals have thus been observed in individuals measuring less than 6 mm nor any

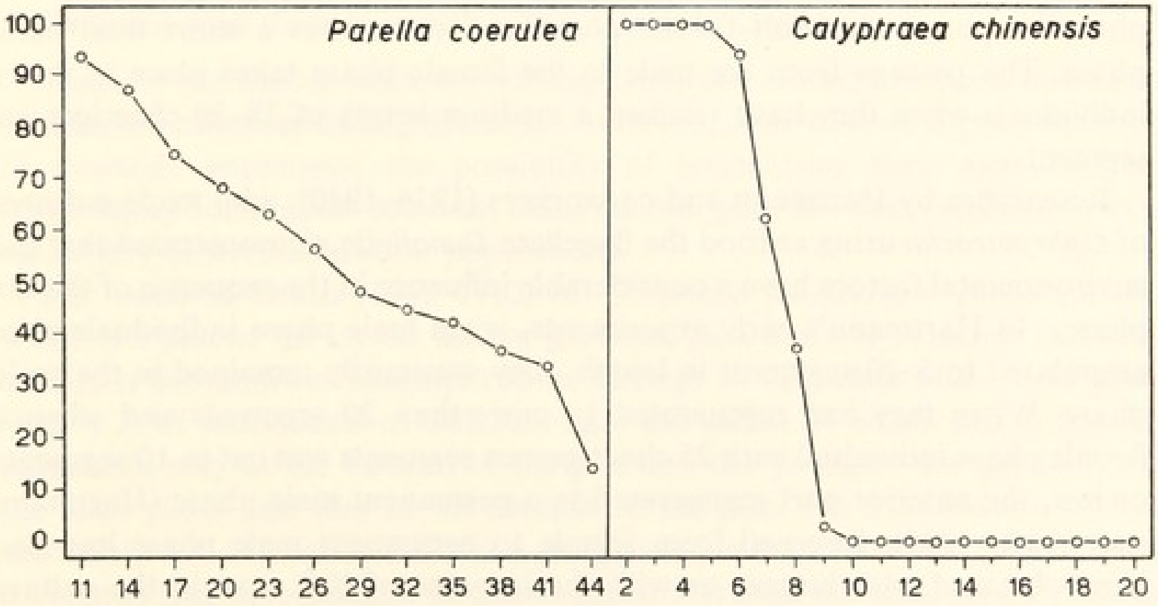

FIG. 7.2. Percentage of individuals in male phase plotted against body length in *Patella coerulea*, an unbalanced hermaphrodite, and in *Calyptraea chinensis*, a balanced hermaphrodite (Bacci, 1947b, 1951).

male phase individual has been found among hundreds of specimens between 10 and 20 mm.

No variability has thus been observed in the sex phenotypes of *Calyptraea chinensis* which is the most rigid example of balanced hermaphroditism. The populations of *Asterina gibbosa* from the Plymouth Sound have been reported as the first example of this type of hermaphroditism (Bacci, 1949a) but their uniformity is not so strict. *Asterina pancerii*, which is a protogynous hermaphrodite (Cognetti, 1954) can also be included in this category with the protandrous nematode species *Coenorabdites elegans* whose cycle has been described by Delavault (1957).

Thus the widespread belief in the uniformity of the sex phenotype in hermaphrodite species has been confirmed only in a few well analysed instances while the examples of extreme variability are now very numerous.

The concepts of balanced and unbalanced hermaphroditism have been introduced in order to point to the importance of the phenomenon of sex variability in hermaphroditic populations rather than in order to establish a sharp division between bisexual species showing high and low sexual variability among bisexual organisms. It must also be pointed out that the distinction is applied to populations and not to single hermaphrodite individuals.

4. Sexual Phases and Environmental Factors in Ophryotrocha

Also the polychaete worm *Ophryotrocha puerilis*, which was first investigated by Braem (1893) and by Korschelt (1893), is a protandrous hermaphrodite species which undergoes a purely male phase, a morphologically hermaphroditic phase, an adult female phase and sometimes a short final male phase. The passage from the male to the female phase takes place in many individuals when they have reached a medium length of 18–20 chaetigerous segments.

Researches by Hartmann and co-workers (1936–1940), who made cultures of *Ophryotrocha* using as food the flagellate *Dunaliella*, demonstrated that the environmental factors have a considerable influence in the sequence of the sex phases. In Hartmann's early experiments, when male phase individuals were amputated to 5–10 segments in length, they constantly remained in the male phase. When they had regenerated to more than 20 segments and when a female phase individual with 25 chaetigerous segments was cut to 10 segments or less, the anterior part regenerated in a permanent male phase (Hartmann and Huth, 1936). Reversal from female to permanent male phase has also been obtained with hunger or with the increase of K^+ ions in the culture liquid (Hartmann and Levinsky, 1938).

Masculinizing substances seem to be produced from ripe female phase individuals: if young unripe females are introduced in the culture medium where ripe adult females have lived for 8–10 days, their development is hindered and their eggs are totally or partially destroyed and reabsorbed until a masculinization takes place (Hartmann and Levinsky, 1940) and the formerly female phase individuals become capable of fertilizing female phase individuals.

It has been subsequently demonstrated (Bacci, 1951c, 1952b) that sex reversal after amputation is not due to rejuvenation but to alterations in metabolism because amputation has no influence on the sex phase if the animals are nourished with *Chlamydomonas*: the female phase can be normally reached by initially male phase individuals that are indefinitely kept at a length of 5 chaetigerous segments by means of repeated amputation.

Müller (1962) has made a re-investigation on the whole series of experiments and has confirmed that masculinization obtained in the Hartmann's experiments is due to a hunger effect. It appears also that reversal to the male phase can only be obtained through contact with the female phase individuals.

Experiments were initiated in order to establish whether the action of environmental factors on the sex phenotype is actually controlled by innate genetic tendencies and it was shown (Bacci, 1952) that masculinization of female phase individuals by ripe females takes place more easily in individuals from the Atlantic than in individuals from the Mediterranean sub-species which, both in nature and in normal laboratory cultures, pass to the female phase at an earlier stage than the Atlantic sub-species (Bacci and La Greca, 1953) and the result was confirmed in similar experiments by Müller. The genetic system which controls such innate patterns of sex differentiation will be discussed in Chapter 8.

5. *Environmental Factors and Sex Phenotypes in* Crepidula

Much of the earlier work on hermaphroditism among molluscs has been done in the extensive fishing grounds of edible species where the great number of available specimens, the possibility of establishing their age and the opportunity for regular seasonal observations gave the first evidence of sex variability in hermaphrodite populations.

The work of Orton (1909–1912) on *Crepidula fornicata*, a prosobranch, which is a pest of the oyster fishing grounds, gave decisive evidence that this species is protandrous hermaphrodite. Groups of *Crepidula* often form piles (Fig. 7.3) of individuals of different ages the oldest of which are in female phase and stay at the bottom of the pile and the youngest are generally in the male phase and stay at the summit of the pile. The examination of the secondary sex characters showed a progressive reduction of the penis and a corresponding development of the so-called uterus in the transition from the smallest to the biggest individuals of each group. The presence in the piles of functionally hermaphrodite individuals having smaller sizes than individuals that are still in the male phase indicate that such individuals have a stronger female tendency.

A work by Wilccynsky (1959) substantially confirms the previous observations by Orton but it points out that female phase individuals are not fertilized by male phase members of the piles but by male solitary visitors.

Coe worked on *Crepidula plana* of the Pacific coast (1948) and went deeper into the analysis of the sex phenotypes.

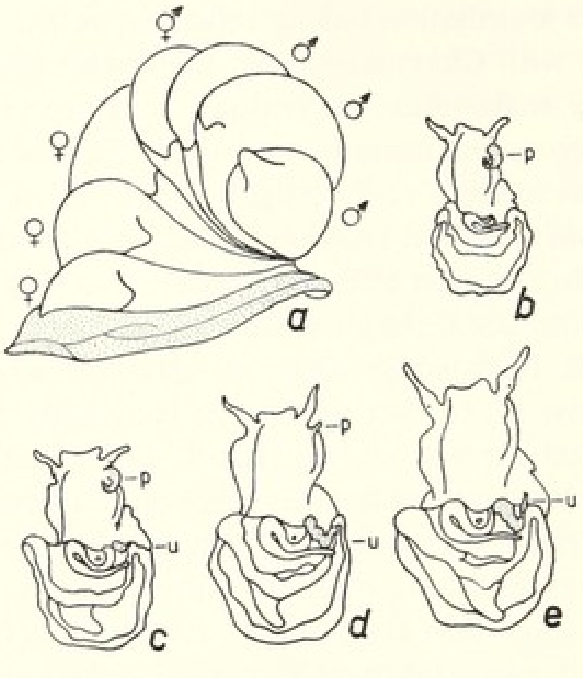

Fig. 7.3. The sexual phases of the hermaphroditic Gastropod *Crepidula fornicata*: a, a pile of seven individuals of different ages attached to an oyster shell; b–c, passages from male to female phase with the gradual reduction of the penis (p) and the corresponding development of the female duct (u) (from Orton, 1912).

A few among the associated individuals are easily identified as pure males because they never pass to the female phase; some associated individuals have, on the contrary, a very short male phase and soon pass to the female phase that has therefore a very long duration. Individuals of intermediate conditions, having male and female phases of almost equal length, form the majority of the specimens that are found associated.

Pure female phenotypes are also found among solitary individuals some of which pass to the female phase after a long period of gonadal inactivity. Such a category has also been detected among artificially isolated specimens.

Pure males are therefore found among the associated individuals, pure females have been detected among the solitary. Both pure males and females reach lengths that remain below the maximum sizes reached by the protandrous individuals, a feature which made the analysis of the sex phenotypes extremely difficult and their interpretation was possible after the results in *Patella* provided a generalized model for unbalanced hermaphrodites.

The associated condition is more favourable to the expression of the male, the solitary condition to the expression of the female sex. This observation on natural population individuals confirms previous results by Gould (1919) who assumed that the female individuals produce hormone-like substances which accelerate male differentiation in unripe young individuals and cause the prolongation of their male activity.

The similar effects of substances produced by ripe females in *Bonellia* made Gould's interpretation very probable although Coe contended that male-producing stimuli are essentially mechanical and that the sexual genotype has an important role in the relative duration of the male and female phase. The conclusion reached by Müller in *Ophryotrocha* (1962) lend new support to Coe's interpretation.

6. *Rudimentary Hermaphroditism in Trematodes*

A good example of rudimentary hermaphroditism is shown by *Wedlia bipartita*, a trematode which is a parasite of birds (Odhner, 1907). Its embryo, which has been developed from a single egg, forms a cyst and from the posterior end of the embryonic body separate two cellular territories that develop as male and female individuals. Each of them is a rudimentary hermaphrodite (Fig. 7.4) because it has the rudiments of the gonad of the opposite sex.

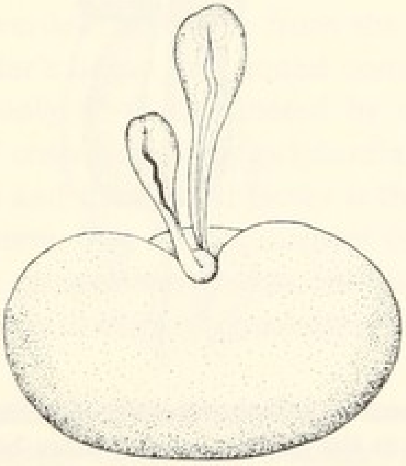

FIG. 7.4. A female and a male *Wedlia bipartita*, a Trematode species showing rudimentary hermaphroditism (Ohner, 1907).

This case represents a passage from a purely hermaphrodite condition to the condition of a gonochoric animal which undergoes "phenotypical sex determination". The situation of *Wedlia* is also the reverse of that which is observed in the monozygotic twins of digametic animals. While such twins have always the same sex in species with digametic sex determination, they have different sexes in *Wedlia* and form together a sort of composite hermaphrodite colony.

A case resembling *Wedlia* has also been observed in *Schistosoma mansoni*, another trematode. Buttner (1950) found that when a host contains only male *Schistosoma* a degeneration of the testes may take place in *Schistosoma* and rudimentary ovaries appear. The example of *Schistosoma* is similar to other mechanisms of sex ratio regulation that shall be considered in this chapter.

Wedlia and *Schistosoma* belong to a hermaphrodite group but rudimentary hermaphroditism has also been observed in the typically unisexual class of amphibia.

7. Rudimentary Hermaphroditism in Amphibians: Bidder's Organs

Two ovarian nodules are present in adult male toads above the two testes and have been known for a long time as Bidder's organs (Fig. 7.5). They can be considered as aborted ovaries that develop from the cranial part of the genital crests and contain well developed oocytes.

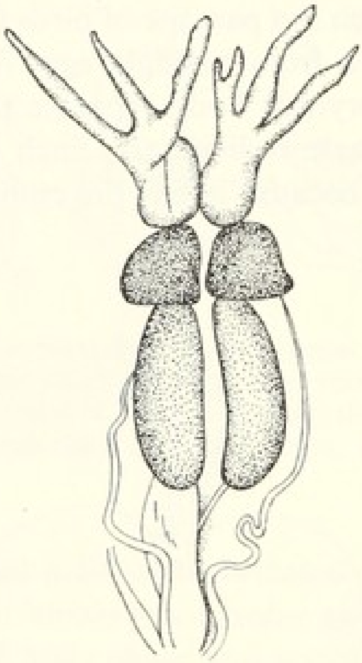

FIG. 7.5. Male genital apparatus of *Bufo* showing the digitated fat bodies above, the Bidder's organs in the middle and the testes below.

The removal of the testes in *Bufo vulgaris* induces hypertrophy of Bidder's organs which develop into big functional ovaries with ripe eggs (Harms, 1921; Ponse, 1925) and the consequent development of oviducts allows us to test the type of sex digamety in toads by fertilizing the eggs laid by such inverted males (Ponse, 1941).

A series of experiments which have been recently carried on by Vannini and by Ghirardelli (1959–1962) provide a clear analysis of the factors affecting the development of the Bidder's organ and of the gonad proper. They represent therefore a possible model for interpretation of the mechanisms that develop rudimentary hermaphroditism in other species.

The genital crest of *Bufo* differentiates at an early stage into a cephalic region that is rich in germinal cells and into a caudal region, which contains less numerous germinal elements. A gradient in crowding of the germinal cells is evident between the two regions and the former develops into the Bidder's organ, the latter forms the proper male or female gonads.

The removal of the cephalic (or Bidderian) tract of the presumptive genital mesoderm leads to the formation of short gonads and of Bidder's organs which originated therefore from the anterior portion of what—in normal development—would have become the anterior part of the gonad. No gonads were formed, on the contrary, after the removal of the caudal (or gonadal) portion and the Bidder's organs normally developed from the anterior region which was left untouched.

The transplantation of the Bidderian tracts of the germinal presumptive mesoderm of an embryo to the gonadic region of the germinal mesoderm of another embryo gave rise to a peculiar formation composed by a Bidder's organ in its normal position, by a gonadal tract, by a second Bidder's organ and by a terminal gonadal tract. The transplantation of the gonadal region of one embryo to the normally Bidderian region of another embryo gave rise on the other hand to a Bidder's organ in cephalic and to a gonad in caudal position. A small gonadal tract is often formed, however, in cranial position within the transplanted territory.

Both Vannini and Ghirardelli conclude from the above experiments that the formation of the Bidder's organ and gonad complex is regulated by two main factors which can only be discriminated by experimental procedure. The first is the gradient of crowding of the primordial germ cells that migrate early into the genital crest and the second factor is the inductive action of the cephalic or caudal mesoderm. The former induces the formation of the Bidder's organ, the latter the gonadal formation and the early establishment of the crowding gradient masks in normal development the inductive influences of the mesoderm.

A developmental analysis of other examples of rudimentary hermaphroditism (which in some instances cannot be distinguished from intersexuality) might likewise be founded on an experimental discrimination of innate potencies from environmental influences.

It can be concluded from the examples given in this section that the sexual phenomena included under the comprehensive term of hermaphroditism are extremely varied. Their phenotypical expression ranges from apparent gonochorism, which is a kind of true hermaphroditism, to rudimentary hermaphroditism, which lies on the borderline with unisexuality and cannot some time be distinguished from developmental intersexuality. Morphological comparison has shown (Pelseneer, 1895) that in some hermaphrodite species eggs and sperms are produced within the same gonadal tubules, in other species they are produced in different but adjacent tubules. Some species show distinct male and female regions of the gonad and some others show distinct male and female gonads in the same individuals. It was remarked (Bacci, 1951a) that such types of classification consider gonadal conditions at the end of their sexual differentiation and therefore resemblances at such stages are often only apparent. A classification of the different types of hermaphroditism from a morphological point of view is therefore unsatisfactory at the present stage of our knowledge.

The distinction between balanced and unbalanced hermaphroditism appears to be helpful at the present time when the sex determining mechanisms of hermaphrodites are still largely unexplored. It helps in understanding the tremendous variety of the sex phenotypes shown by the different species on the basis of the study of sex variability within single hermaphrodite species or populations.

The *Bonellia* Case

Bonellia viridis is the best-known example of the influence of environmental factors on sex differentiation in unisexual animals. It is a marine echiurid worm which shows a marked sexual dimorphism. The body of the female (Fig. 7.6) reaches the size of a walnut and it is provided with a long lobated proboscis which, when extended, may reach the length of 80 cm. The male is much smaller, from 1 to 3 mm in length and a group of two or three males live on the proboscis or on the uterus of the adult female. From the fertilized eggs are produced sexually undifferentiated larvae which resemble the typical

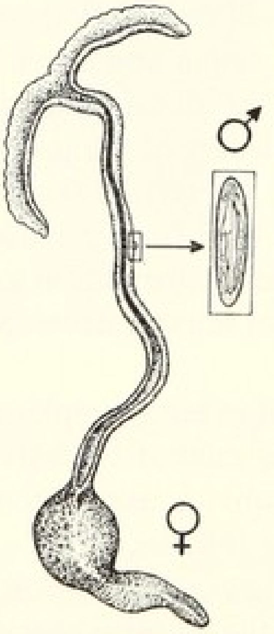

FIG. 7.6. Female and male individuals of *Bonellia viridis*, a striking example of sex dimorphism in a species whose sex is extremely labile during the larval stage (after Baltzer).

trocophora and swim around the rocky bottom where the parents live (Fig. 7.10).

Baltzer showed (1914–1937) that, if the larvae settle on the proboscis of a ripe female, they develop into males, if on the contrary they settle far from the adult female individuals they differentiate into females. If the larvae that have settled on the proboscis are removed after a short period and they are compelled to develop in pure sea water, intersexes are obtained. Their male characters will appear the more marked the longer the permanence upon the female body. Isolated larvae, which are kept in an aquarium, generally develop into females but if pieces of a proboscis are put in the aquarium the larvae are developed into males. The same male producing action is obtained by employing water extracts of the proboscis and of the intestine (Baltzer, 1926; Nowinski, 1934).

A high percentage of male individuals was also obtained from free living larvae with the addition of inorganic substances to the pure sea water of the cultures (Herbst, 1928–1936). Addition of CO_2, of HCl, of K^+ and traces of Cu^{++} produced from 60 per cent to 92 per cent male individuals. Also the subtraction of SO_4'' and of Mg^{++} ions from the sea water induced the differentiation of about 90 per cent *Bonellia* larvae into males.

Sex determination appears thus to be purely phenotypical to various authors (Goldschmidt, 1931; Hartmann, 1937) but Baltzer (1932) pointed to the remarkable fact that a few *Bonellia* larvae differentiate as males also in the absence of ripe females and of their extracts and on the other hand some larvae do not attach themselves to the female proboscis when present and therefore they develop as females. The situation of these male and female larvae seems therefore to be very similar to that of pure males and females in the *Patella* case (Bacci, 1947b).

The peculiar example of sex determination in *Bonellia*, which assures the production of two stable sexual states in the absence of a sex chromosome mechanism, provides an excellent example of sexual adaptation to the peculiar condition of a sessile animal.

The sex determination in parasitic animals, which so far has been only tentatively explored, provides an impressive example of sexual adaptation.

Sex and Parasitism

1. Ione thoracica

Ione thoracica is a species of isopod which lives as a parasite on the gills of *Callianassa laticauda*, a decapod crustacean. Reverberi and Pitotti (1942) demonstrated that the isopod reaches the gills of the host in the sexually undifferentiated stage of copepodite larva. The first larva that reaches the gill of *Callianassa* always develops as a female and the second larva differentiates into a dwarf male that lives attached to the female body (Fig. 7.7). Evidence has been collected that developed females exert a male producing action upon the undifferentiated larval stage and also upon female individuals in the early stages of differentiation (Reverberi, 1947) which can be changed into males.

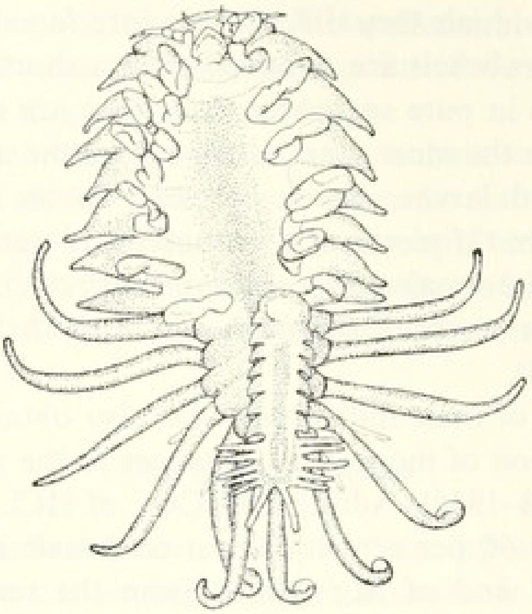

FIG. 7.7. *Ione thoracica*: female with dwarf male (from Reverberi and Pitotti, 1942).

Similar processes were later observed also in the isopod *Stegophryxus*, which is a parasite of *Pagurus* (Reinhard, 1949) and it is probable that a similar mechanism determines the appearance of the dwarf males that have been described in other parasitic isopods (Giard and Bonnier, 1887). The sex determining mechanism of *Ione* closely resembles that of *Bonellia* but larval individuals that are not determined by environmental conditions have not been detected in *Ione*.

Therefore the influence of ecological conditions appears to be even stronger than in *Bonellia* and the adaptation to parasitic life is evident because it always ensures the presence of both sexes when only two individuals are present in the host.

2. *Paramermis contorta*

The nematode *Paramermis contorta* is a parasite of *Chironomus* larvae and sex appears on the whole to be correlated with the number of parasites that are found in a single larva (Caullery and Comas, 1927): if they are numerous they are mostly males and they are almost always females if they are isolated. A small percentage of individuals differentiate nevertheless as males also when they are isolated in a single larval *Chironomus* and a female individual has also been found in groups of nine or ten male individuals living in a single larva of *Chironomus*.

The similarity with the *Bonellia* and *Ione* cases appeared evident and

Parenti has resumed the investigation (1961–65) by introducing different numbers of parasites into *Chironomus* larvae at different developmental stages. It has been established that the proportion of male *Paramermis* increases with the number of parasites that are introduced in each larva and that it decreases with the length of the larvae at the time of introduction.

A large group of 11-days-old *Chironomus* larvae was infected with newly hatched *Paramermis* and the parasites were sexed 15 days later. A second group of *Chironomus* larvae was subjected to a first infestation at 2 days of age and to a second infestation 9 days later, that is at the same age of the *Chironomus* larvae of the first group. The parasites of the second group were also sexed 15 days later and it was possible to distinguish the *Paramermis* from the first (aged 24 days) from those from the second infestation (aged 15 days). Only the *Paramermis* which were found in *Chironomus* larvae occupied in the course of the first infestation by a single parasite were taken into consideration. They were 7941 and they could be compared, with regard to sex proportions, to the 6595 parasites arising from the single infestation in the first group, which were also aged 15 days. 94·6 per cent male *Paramermis* were found among the parasites of the second infestation (N = 1581) which were associated with a single female from the first infestation, 84·5 per cent males were found in the first group (N=6595) and only 61·8 per cent male *Paramermis* were among the parasites of the second infestation (N=6360) which were associated with a single male from the first infestation.

The above experiments show that the proportion of female individuals is much higher among the younger parasites that had penetrated a *Chironomus* which contained already a single male *Paramermis* than it is among those that had penetrated a larva which carried a single female parasite. Sex proportions were also significantly different from those of the controls.

The *Paramermis* research has thus established that males exert a female determining influence upon undifferentiated individuals and that females exert a complementary action (1965).

As female determining influence of the males has been demonstrated so far only in *Paramermis* but it is likely to be found in other parasitic organisms.

The sex determining action of the environment is nevertheless not so rigid in *Paramermis* as it is in *Ione*. Parenti established on the other hand (1961) that responses to environmental conditions are different in different populations, a result which closely resembles those obtained in the different races of *Ophryotrocha*.

The variability in the response to the environmental sex determining factors is higher in *Paramermis* than in *Bonellia*, and it represents a very delicate adaptation to the peculiar type of parasitism in *Chironomus* where a great number of *Paramermis* may be found in a single larva. The possibility of producing even a single female in a numerous colony of parasites makes possible for such group to have a progeny even if the prevailing environmental influences may lead to determination in the male direction.

3. Mytilicola intestinalis

Mytilicola intestinalis is a Copepod which lives as a parasite in the intestine of the edible mollusc *Mytilus galloprovincialis*. The sex ratio of this copepod has been studied in three populations from the fishing grounds of Porto Corsini, in the Adriatic, near Ravenna, and from Olbia and La Spezia in the Tyrrenian sea (Bacci *et al.*, 1958). The general conclusions to be drawn, from the study of nearly two thousand parasitic adult individuals in the three populations are the following. The percentage of the males is about 44 per cent when a single *Mytilicola* lives in the intestine of *Mytilus*, the 1 : 1 sex ratio is reached when two *Mytilicola* only live in the intestine and the percentage of males gradually reaches the maximum value of about 70 per cent when the number of six parasites per *Mytilus* is reached. Minor differences in the change of sex ratios are now being investigated inside each *Mytilicola* population in different fishing grounds.

A similar situation has been found in the potato root eelworm *Heterodera rostochiensis* that shows higher proportion of males in the most heavily attacked parts (Ellenby, 1954) and the male determining factor is unknown although hypotheses based on the action of food shortage, of waste substances and of hormone-like substances may be advanced.

A decreasing influence of sex determining action of environmental factors is observed in the above examples which form a series beginning with *Ione* and ending with *Mytilicola*. The environmental action is expressed in the latter with a significant but slight preponderance of the female sex when a single parasite is present in *Mytilus*, and with the gradual increase of the percentage of male individuals with the increase of the degree of infestation. The isolated parasites are almost exclusively females in *Paramermis* and a very rapid increase in the number of males with the increase of the number of parasites per *Chironomus* larva is observed. In *Ione* not a single exception has been observed to the existence of female phenotypes when the parasite is isolated on the gills of *Callianassa*.

It appears evident that the different responses of the three species to the environmental actions show a strict adaptation of the sex determining mechanisms to the peculiar types of the host-parasite relationship.

4. Sexual Phases and Parasitism in Cymothoida*land the Problem of Dwarf Males*

Very little experimental work has been done on sex determination in hermaphrodite parasite species although hermaphroditism is considered one of the most obvious means of adaptation to parasitic life.

Montalenti (1941) made a study of the gonad differentiation and development of secondary sex characters of hermaphrodite Isopods of the family Cymothoidae that live as external parasites upon teleosts of different species. Larval gonads of *Emetha audouini* and of *Anilocra physodes* show the primordia of both testicular and ovarian parts and the parasites develop in the male phase soon after they have become fixed to the host fish. A few oocytes are already well differentiated when sperms are produced and secondary sex characters are still of male type. When the activity of the testicular lobes is reduced the germinal elements in the ovarian region ripen. Female secondary sex characters soon make evident the change of sex phase.

Breeding experiments which were subsequently made on *Anilocra* by Legrand (1952) have shown that male phase individuals living with a female* change to the female phase later than isolated male phase individuals. Such associated males do not pass to the female phase until the female partner has been eliminated. Transition stage individuals change to the female phase earlier when they are associated with males than when they are kept isolated. These results are comparable to those obtained by Gould and by Coe in *Crepidula* although the variability of the sex phenotypes is not so high in *Anilocra* as in *Crepidula.*

Sex reversal has more recently been obtained in *Anilocra* through the implant of the androgenic gland in a female phase individual (Legrand and Jouchault, 1961).

The prolongation of the male phase, which results in the presence of big male individuals, and the shortening of the transition stage are due in Legrand's interpretation, to the exchange of stimuli between the mating pair and the existence of a masculinizing substance, having an action similar to that demonstrated in *Bonellia,* is considered doubtful.

The work on cymotoids gives some ideas concerning the solution of the old problem of the dwarf complementary males. They have been described by Darwin (1851) in the sessile and hermaphrodite Cirripedia and dwarf males that retain larval organization have also been recently studied (Deroux, 1961) in *Montacuta phascolionis,* a bivalve which lives on *Phascolion.*

In *Peltogaster paguri,* a rhizocephalian cirriped, which is parasite of a hermit crab, cypris larvae gather in the so-called mantle cavity of an adult hermaphrodite individual and, according to Reinhard (1942), are transformed into neothenic males which add their sperm to the sperm produced by the male germinal elements of the hermaphrodite individual. The factors inducing masculinization of the indifferent cypris larvae are unknown although the resemblance to the *Ione* case is striking.

It appears possible that complementary males of other cirripeds, and possibly of bivalves, are developed in a similar way although it can be also assumed (Bacci, 1947b) that they may be genetically assimilated to the pure

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* Hermaphrodite individuals in male or female phases are often simply indicated as males or females.

males of unbalanced hermaphrodite species. It is evident that the decision between the two hypotheses can only be obtained through breeding experiments.

Sex and Symbiosis

Buchner (1954) has described a case of sex determination where the development into female or male individuals seems to be induced by the early infection of the egg by symbionts from the mycetome. In *Stictococcus sjoestedti*, a species of coccid of the tropical regions, the female individuals carry symbionts and the rudimentary males are free from symbionts. Sections through the ovaries of ripe females show that when the fertilized oocytes have reached the diameter of about 50μ they come in contact with the mycetocytes that lie in the periphery of the ovarian region. If the symbionts penetrate the egg (Fig. 7.8) the zygote differentiates into a female individual, and it differentiates into a male if it is not infected. The female and the male producing embryos are therefore recognizable at early stages from the presence of or the absence of symbionts (Fig. 7.9).

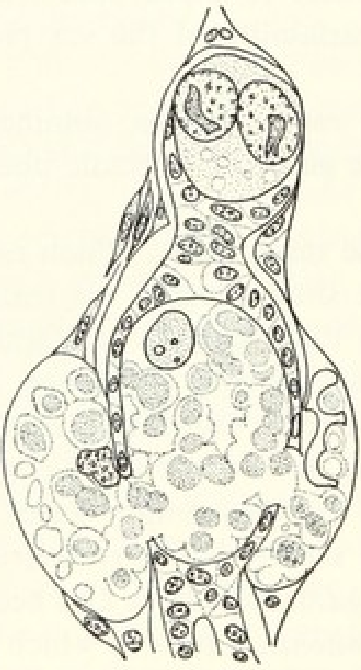


FIG. 7.8. The eggs of the Coccid *Stictococcus sjoestedti*, which become infected by symbiotic fungi from the mycetome while entering the ovarian chamber, give rise to female individuals, otherwise they form male individuals. The infection of an egg is shown (Buchner, 1954).

The position of the oocytes in the ovary decides thus whether they become infected or not from the mycetocytes and substantially whether a female or a male individual is produced. The variations in sex ratio which have often been observed among coccids and especially the high number of males in the progeny of old female individuals (Hughes Schrader, 1948) may thus be due to irregularities in the mode of infection from mycetocytes. No information is available at present on the chromosome constitution of *Stictococcus*.

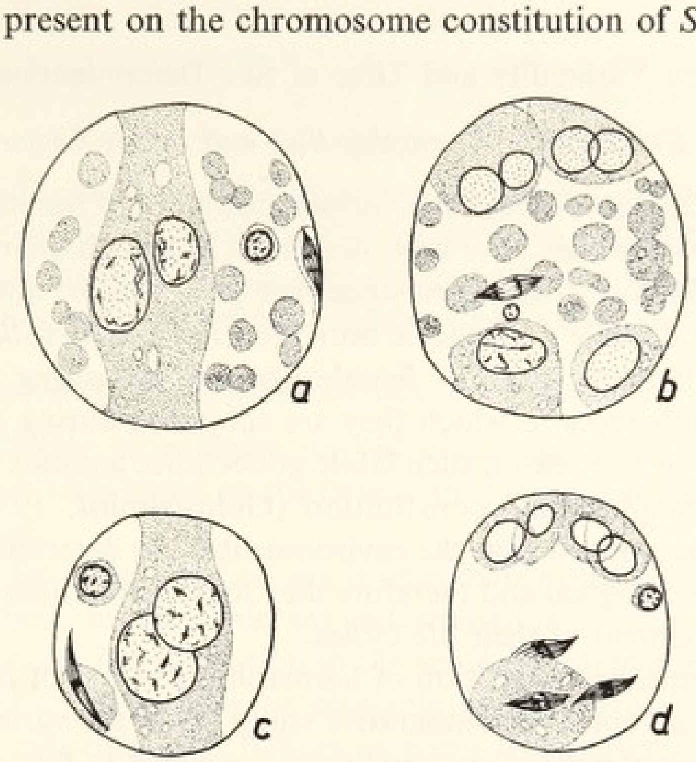


FIG. 7.9. Two stages of development of symbiont infected eggs, one (a) showing the two pronuclei and the other (b) at four cell stage, are compared with stages of non-infected eggs (c and d), which will form male individuals (Buchner, 1954).

Lampel (1958) has discovered a different behaviour regarding the influence of symbionts in the aphid *Pemphigus spirothecae* which lives on a single plant. Male and females of the amphigonic generation (the so-called *sexuales*) are produced parthenogenetically by a single female (a mictic sexuparous female). Each mictic female generally produces six eggs only. Two of them assume a cranial orientation and they will become males, the remaining four take a caudal position and they will become females. The two cranial eggs will not become infected by symbionts and the remaining four eggs are infected and differentiate into females. Thus the sex determination is obtained in *Pemphigus spirothecae* through the position of the eggs and the introduction of the symbionts seems to have a secondary influence in sex determination.

Lampel (1958) could discover no sex determining action of symbionts in another species of *Pemphigus*, but sex appears to be determined there by the sex chromosome mechanism which will be illustrated in a discussion of the heterogonic cycles in aphids.

It is possible to conclude that adaptation to symbiosis implies sex determining mechanisms where the influence of infection with symbionts seem to be predominant and mechanisms where, as in *Pemphigus*, the inherent patterns of development have taken control of sex determination.

Sex Variability and Time of Sex Determination

1. Balanced and Unbalanced Hermaphrodites and Labile Gonochorists

The unifying feature between the various examples of hermaphrodites and of labile gonochorists that have been described in this chapter is represented by the fact that hermaphrodites produce ripe germ cells of both sexes during their life cycle and labile gonochoric individuals of the *Bonellia* type are able to produce indifferently male or female gametes according to the natural environmental influences to which they are subjected during given stages of their life cycle. For this reason such labile gonochoric animals have been said to have a "hermaphrodite" constitution (Goldschmidt, 1931; Hartmann, 1943). It must be noticed that the environmental sex determining factors in such species are ecological and therefore they must be considered as a normal and integrated element of their life cycles.

Once this orientative unification of hermaphrodite and of labile unisexual species has been accepted, a comparative study of the sex variability is useful in the extreme complexity of sex conditions examined in this chapter.

Hermaphrodites have been classified into two types:

(a) The *Patella* type (or unbalanced hermaphroditism), which represent the pole of extreme sex variability.

(b) The *Calyptraea* type (or balanced hermaphroditism) which is the example of the uniformity of the sex phenotype.

This classification applies to hermaphrodite populations and therefore it is derived from the type of sexual differentiation or alternation of the sex phases that takes place in the single individuals of the population. It is not important from this point of view whether the species is a protandrous or a protogynous hermaphrodite, whether it shows false gonochorism or it is a simultaneous hermaphrodite, whether male and female germinal elements are produced in the same follicle or in separate gonads of the same individual. Cognetti and Delavault (1960) have also shown that unbalanced hermaphroditism can be demonstrated in species where individuals undergo a series of alternate male and female sex phases, as in some populations of *Asterina* or in some oyster species.

When the sexually labile gonochoric species are taken into consideration it is possible to demonstrate considerable differences among them because in some species or populations all the individuals react in the same way to the environmental conditions (as in *Ione*) and in others (as in *Bonellia*) there is a considerable variability in the individual reaction to the ecological sex inducing factors. For this reason the *Bonellia* larvae that develop into males even when isolated and the *Bonellia* larval individuals that do not become fixed to the female were seen to be similar to the pure males and females from *Patella* (Bacci, 1947b). The *Paramermis* case gives a good example of such variability. It is possible to apply the criterion of the variability of sex phenotypes to the various examples of sexually labile gonochorists.

The preliminary examination of numerous samples is necessary for the study of sex determination of hermaphrodite or labile gonochoric species as the problems concerning the inheritance of the sexual genotypes appear to be quite similar in unbalanced labile gonochoric populations and in unbalanced hermaphrodites in spite of their quite different patterns of sexual development.

2. *Progamic, Syngamic and Metagamic Sex Determination*

It is now possible to examine an old classification of the types of sex determination which is based on the time of the life cycle when the sex appears to be determined.

In progamic sex determination sex is irreversibly established in the oocyte before fertilization, in syngamic sex determination (which roughly corresponds to genetic sex determination in the sense of Hartmann) sex is fixed at fertilization and in metagamic or epigamic sex determination sex is firmly established by environmental factors at some stage after fertilization or it remains in a labile state subject to the influences of ecological factors.

It is clear at present that fertilization is always the decisive event for the determination of sex in the diploid organisms, even if sex is not irreversibly determined when caryogamy takes place, because the reaction norm of the sex phenotype to environmental factors is fixed at fertilization. It has been shown that in the well-known case of *Dinophilus* the sex ratio is very likely determined by the maternal genotype.

The classification proposed by Haecker is therefore important from a strictly embryogenetic point of view because it points to the time when the integration of genetical with environmental factors leads to the irrevocable determination of the individual sex phenotype according to the specific patterns of differentiation, or it leads to the determination of a labile sexual phase.

An examination of a few cases that were formerly included in the category of the phenotypically determined organisms will elucidate this point (Fig. 7.10).

In *Dinophilus* the moment of sex determination corresponds to the growth period of the oocytes prior to fertilization and even to the maturation period when the maternal genotype fixes the ratios of the male–female progeny. A similar case is presented by *Stictococcus* whose sex is fixed in unfertilized oocytes through the penetration of the mycetocytes. Determination takes place in *Bonellia* a little after the larval stage when it becomes impossible to reverse the sex phenotype. The transition to the female phase is not possible in most *Ophryotrocha* until a length of 18–20 segments is reached but the sex expression remains labile because ecological environmental factors easily induce masculinization.

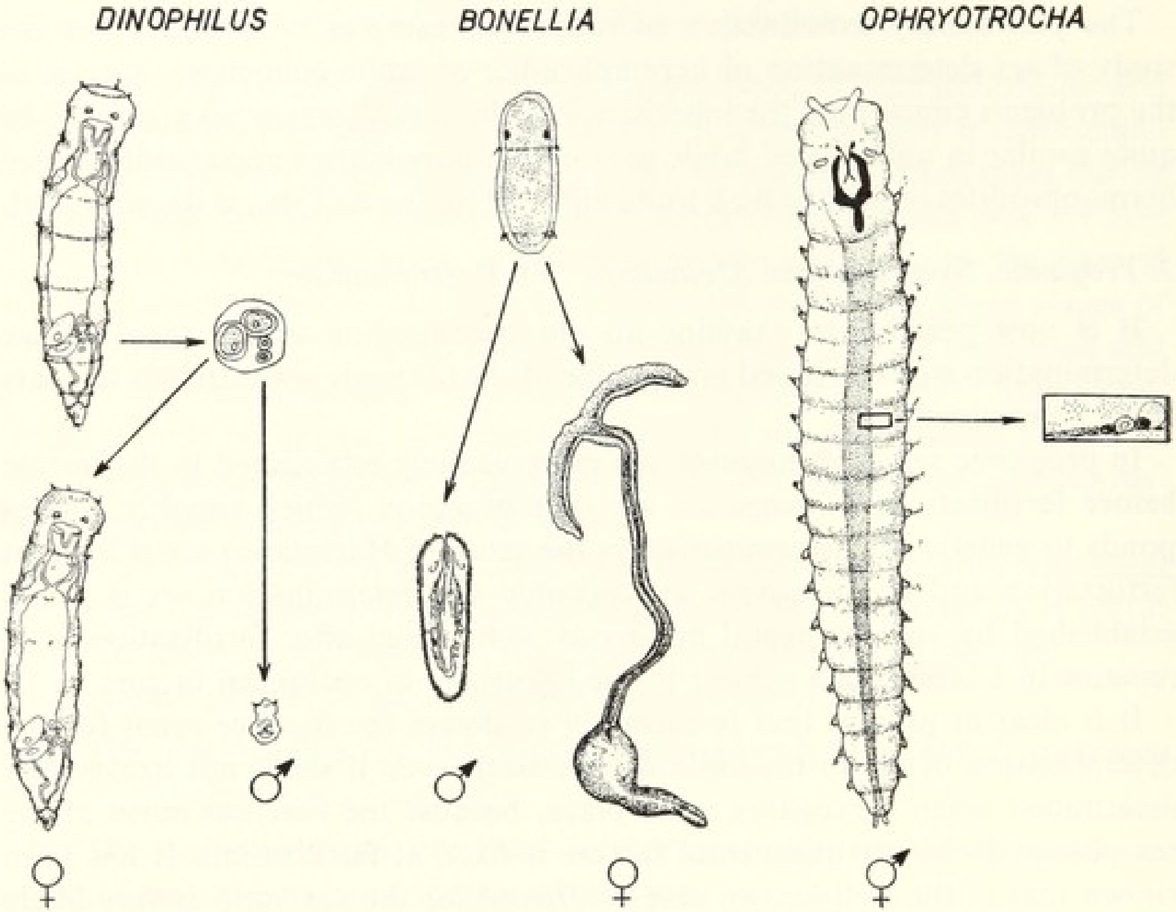


FIG. 7.10. Diagram showing the times when sex is determined: in *Dinophilus* sex is determined in the developing oocytes before maturation divisions, in *Bonellia* sex is determined soon after larval stage, in *Ophryotrocha* sex determination is postponed to adult stage.

The individuals of unisexual species, like *Drosophila* or *Xiphophorus*, are sexually determined at zygote formation under normal conditions prescinding whether their determination is digametic or polygametic.

It is important therefore to distinguish between the patterns of sex differentiation concerning the time when sex is embryologically fixed and the patterns of sex variability, preliminary knowledge of which is necessary in order to understand the mode of inheritance of the genetic factors in hermaphrodites and in labile gonochorists.

3. The Problem of Sex Variability

It is now possible to discuss again the FFMM formula that has been adopted with minor variations by the earlier authors to interpret the situation of sex genes in diploid hermaphroditism and in the gonochoric species that were regarded as constitutional hermaphrodites.

It appears reasonable to assume that male and female sex determining factors are perfectly balanced in hermaphrodite individuals that produce male and female gametes during male and female phases of equal duration and in unisexual individuals that can be differentiated with the same facility by ecological factors either into males or into females. There appears to be no difference, with regard to sex balance, between hermaphrodites and such gonochoric species. The FFMM formula thus roughly expresses this balanced situation of sex factors whose action produces equivalent expressions of male and female sex characters in balanced hermaphrodites and equal possibility of differentiation in the male or in the female direction in labile gonochorists.

The formula appears, however, unsatisfactory when unbalanced hermaphrodite populations or unbalanced labile gonochorists are taken into consideration and the question was early raised by Baltzer (1937) who doubted the hermaphrodite constitution of *Bonellia* at a time when the study of sex variability in hermaphrodites was ignored and it was raised again when the *Patella* case of sex variability was investigated (Bacci, 1947).

The question is to establish whether all the members of unbalanced hermaphrodite populations have the same sex genotype with differences in sex phenotypes that are determined only by environmental factors or they have different sex genotypes. It must be established in other words whether only sex monogamety or also sex polygamety is present in the known examples of hermaphroditism and of labile gonochorism.

CHAPTER 8

HERMAPHRODITISM, BALANCE AND MODIFICATION

GENETIC investigations on sex determination of hermaphrodite animals are unfortunately very scarce at present. If, however, the extremely varied sex phenotypes of hermaphrodite species are to be understood in terms of adaptation to parasitism, to sessile life or to the multiple possible peculiarities of their cycles, a preliminary understanding of their sex determining mechanisms is necessary. Such knowledge will also prove very important in the frequent cases of gonochorist groups which are phyletically related to hermaphrodite groups (Bacci, 1947, 1950; Cognetti and Delavault, 1960) because hermaphroditism, as well as labile gonochorism, does not form a category which is genetically isolated from the category of sex digametic species.

The available information on sex variability among monoecious plants is also scarce although monoecism represents a primitive condition in angiosperms. Some investigation has, however, been carried on on plant species which show some degree of sex variations or have undergone mutations concerning the male or the female organs. They are very significant and will be discussed before approaching the genetic problem of unbalanced hermaphroditism in animal species.

The Case of *Ecballium elaterium*

Galan (1946) discovered that the populations of *Ecballium elaterium* of the northern half of Spain are monoecious and that most of them are dioecious in the southern part of Spain. Crosses between dioecious and monoecious plants and their hybrids led the author (Galan, 1950, 1951) to the conclusion that the two conditions are determined by a series of multiple alleles a, a^D, a^d, where *a* is the initial of androgynous, and D indicates the dioecious condition.

The effects of the different combinations are the following:

$a^D\ a^d$ = ♂ of the pure *dioicum* race
$a^+\ a^D$ = ♂ hybrid
$a^+\ a^+$ = ⚥ of the pure *monoicum* race
$a^+\ a^d$ = ⚥ hybrid
$a^d\ a^d$ = ♀ of the pure *dioicum* race

All the experiments support this interpretation which implies also that what, in Westergaard's terminology, might be called the male sex deciding gene is carried by the Y chromosome. The allele for the monoecious condition is carried by chromosomes that must be considered as homologous to the sex chromosomes of the dioecious race, a situation that has some analogy with that found in the latent hermaphrodite fish *Xiphophorus helleri.*

Galan's interpretation is fully satisfactory from a formal point of view but it does not provide any clue regarding the evolution of dioecious from monoecious species. The hypothesis of the mutation of an hermaphrodite to a female sex realizator might be advanced in accordance with the similar interpretation of Lorbeer in *Sphaerocarpus*, but the situation is certainly far more complicated.

The female potency of a^d appears to be higher than that of a^+ as resulting from the following proportions of male, hermaphrodite and female flowers in two different genotypes containing a^+ and a^d : a^+a^+ homozygotes show 32 per cent male, 63 per cent hermaphrodite and 0·5 per cent female flowers, while a^+a^d heterozygotes show 25 per cent male flowers, 25 per cent hermaphrodite flowers and 50 per cent female flowers. Selection for prevalently male or female phenotypes might perhaps provide a satisfactory interpretation for the differences in the male and female potencies, and show the existence of an underlying polygenic system of sex determiners.

Hofmeyr (1938, 1939) and Storey (1953) made crosses between hermaphrodite and dioecious strains in species of the genus *Carica.* An allelic series *m*, M_1 and M_2 has been postulated *mm* being female, M_1m male and M_2m hermaphrodite. This interpretation and Galan's interpretation of the *Ecballium* case are very similar indeed.

Sex Variations in *Cucumis sativus*

Four basic sex forms have been recognized by Shifriss and Galun (1956) and by Shifriss (1961) in *Cucumis sativus*, a predominantly monoecious species which shows wide variations in sex expression ranging from almost male to female phenotypes.

The following main sex phenotypes have been described by Shifriss:

1. Monoecious varieties are typically protandrous with production of staminate flowers in the early male phase, with production of both staminate and pistillate flowers in the mixed and of only pistillate flowers in the female phase (Fig. 8.1 A). The beginning of the mixed phase, or first turning point (T1), represents a useful mark for the measure of the sex tendency by counting the average number of leaves from the cotyledons to the first pistillate flowers.

2. Gynoecists (Fig. 8.1 B) occur in races from Japan and Korea and crosses

between gynoecists and monoecists with a high turning point produce intergrade monoecists.

3. Andromonoecists (Fig. 8.1 C) are marked by an early male phase followed by a mixed and by a hermaphrodite phase with perfect or hermaphrodite flowers.

4. Hermaphrodites (Fig. 8.1 D) have been obtained from crosses between andromonoecists and gynomonoecists.

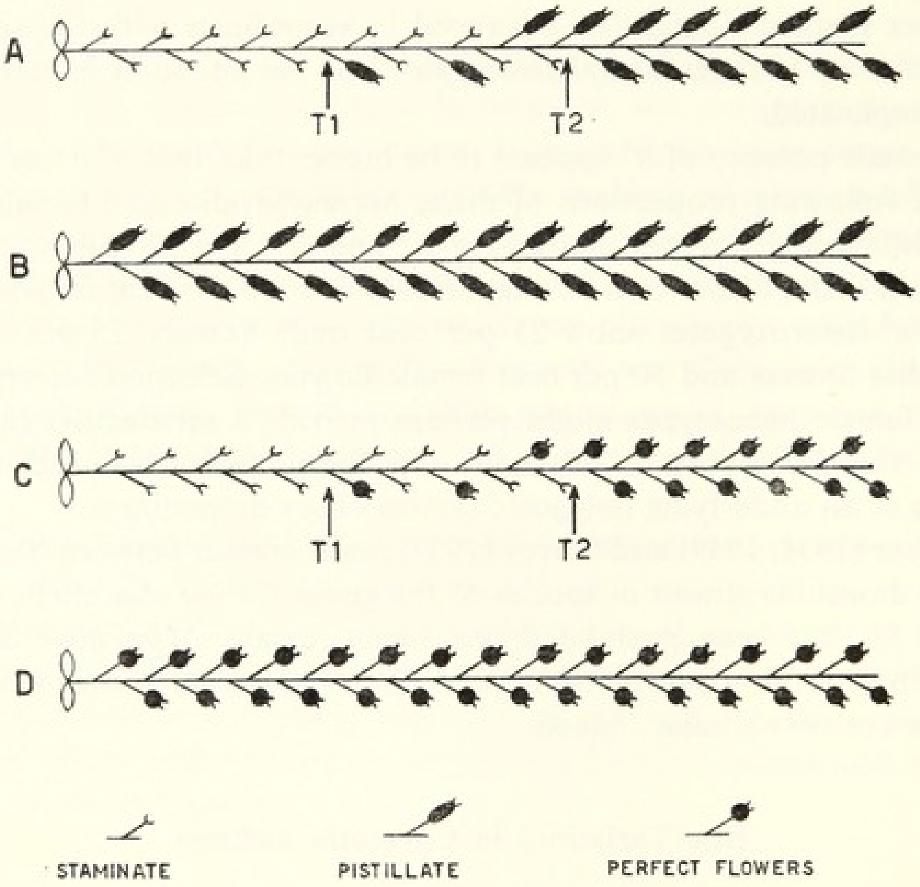


FIG. 8.1. Sex phenotypes of *Cucumis sativus* A, a monoecious variety; B, gynoecious variety; C, andromonoecious variety; D, hermaphrodite variety (Shifriss, 1964).

Interpretations regarding the inheritance of sex expression in the cucumber are given in papers by Shifriss (1961) and by Galun (1961) who seem to have initially investigated upon the same strains.

The kind of flowers present in the four above-mentioned sex forms are determined by what Shifriss calls qualitative genes; that is gene *G* for pistillate flowers, which is present in monoecists, and gene *g* for perfect or hermaphrodite flowers which is carried by andromonoecists. The potentiality for developing staminate flowers is presented—according to Shifriss—by all cucumber plants.

Shifriss reports that monoecious inbreeds showing relatively weak, moderate and strong male tendency were obtained through self-fertilization for at least fifteen generations and the sex tendency was measured by the average leaf number to both turning points T1 and T2 marking the beginning and the end of the mixed phase. It was demonstrated that monoecists that carry genes for a weak male tendency can be transformed into stable gynoecists by a single dose of the G factor, two doses of the G factor were necessary in order to transform moderate monoecists into stable gynoecists and strong male monoecists could not be transformed into gynoecists. Thus polygenes for what Shifriss calls the sex tendency clearly condition the expression of the gene associated with gynoecism. It appears on the other hand that the low percentage of hermaphrodites obtained in the F_2 generation of crosses between gynoecists and andromonoecists is due to the moderate and strong male tendency of the parents employed in the crosses.

In the interpretation of Shifriss the gene *g* associated with gynoecism is carried also by hermaphrodites. A major gene *Acr* (accelerator) seems to accelerate the rate of sex conversions and is associated both with gynoecism (Poole, 1944) and with hermaphroditism. In strong male backgrounds it develops into sub-gynoecists and sub-hermaphrodites of varying degrees.

According to Galun sex expression is affected by two major genes (basically corresponding to the qualitative genes mentioned by Shifriss), one of which is indicated by *st* and induces gynoecism when in double dose. The other is the gene *m* which induces andromonoecism in monozygous condition and has a male tendency. The *M* allele stands for monoecism.

Shifts both in the male and in the female direction were obtained through eight generations of selection from a monoecious stock. A polygenic control of the extension of the male or female flowers was thus demonstrated also in Galun's crosses.

A marked influence of environmental factors upon sex expression has also been demonstrated by numerous observations and experiments. High temperature and long exposition to daylight induce shifts in the male direction (Nitsch *et al.*, 1952). The same effect is produced by treatment with gibberellic acid (Wittwer and Bukowac, 1958) and a shift to femaleness is induced by indole-acetic acid and by α-naphthole-acetic acid. Galun and co-workers obtained *in vitro* (1962) a complete feminization of twelve floral buds out of thirteen which were grown in culture media with the addition of indolyl-3-acetic acid at a concentration of 0·1 mg/l. Such buds would have become male flowers if left in their normal positions in the plant. A complete sex reversal has therefore taken place.

The differences between the two formal interpretations that have been advanced to interpret the inheritance of the sex expressions in *Cucumis sativus* seem not to be very important and they can possibly be interpreted in terms of actual genetic differences existing between the stocks investigated by Shifriss and those investigated by Galun. The work on the cucumber is very important because it has demonstrated the existence of multiple sex genotypes and also a balance or interaction between major and minor sex genes or modifiers which gives rise to the multiple sex phenotypes. The modifying influences of the environmental conditions concur to the variability of the sex phenotypes.

Cucumis sativus is basically similar to *Ecballium elaterium*, which belongs to the same family of Cucurbitaceae, in its mechanism of sex inheritance. Only geographic sex variability was available for investigation in *Ecballium* whereas the enormous variability shown by cultivated strains allowed a far deeper analysis of sex determination in *Cucumis*.

The type of sex determination shown by the Cucurbitaceae is by no means isolated as Janick and Stevenson provided strong indications that sex is determined in *Spinacia oleracea* by three alleles and that modifying genes shift monoecious plants toward maleness or femaleness. A three allele hypothesis was later adopted by Montalenti and Vitagliano (1963) in interpreting monogeny of *Asellus* (see p. 129).

It is worth mentioning lastly that the first observations and experiments demonstrating an influence of multiple sex genes and modifiers in plants have been carried out on *Urtica caudata* (Negodi, 1929) but they are unfortunately still incomplete.

Silkless and Tassel Seed in Maize

The maize (*Zea mais*) is a normally monoecious plant whose terminal inflorescence (the tassel) is male and the lateral (the silk) is female. Emerson (1932) and Jones (1934) discovered mutant autosomal genes which cause the production of prevalently male or female plants.

The recessive allele ts (tassel seed) causes the production of female flowers in place of the tassel. Therefore the plants which are homozygous for such a gene are predominantly female although some pollen may also be produced in the tassel.

The silkless gene (sk), which is located in another chromosome, induces the sterility of the silks when in homozygous condition while the tassels conserve completely functional anthers. Thus predominantly male plants are formed. Plants with the sk sk ts ts genotype are females that show both lateral and terminal flowers. When they are crossed with silkless functional males heterozygous for ts (sk sk Ts ts) they produce males and females exclusively and give rise to a dioecious stock.

A formal identity can be observed between the formula of the silkless males heterozygous for tassel seed (*Ts ts sk sk*) and the formula for *Drosophila* males (FfMM). The formula of double recessive females (ts ts sk sk) is also superposable to the formula for female *Drosophila* (FFMM). Thus the chromosome I of *Zea mais*, where ts is located, can be assimilated to a sex chromosome (Fig. 8.2).

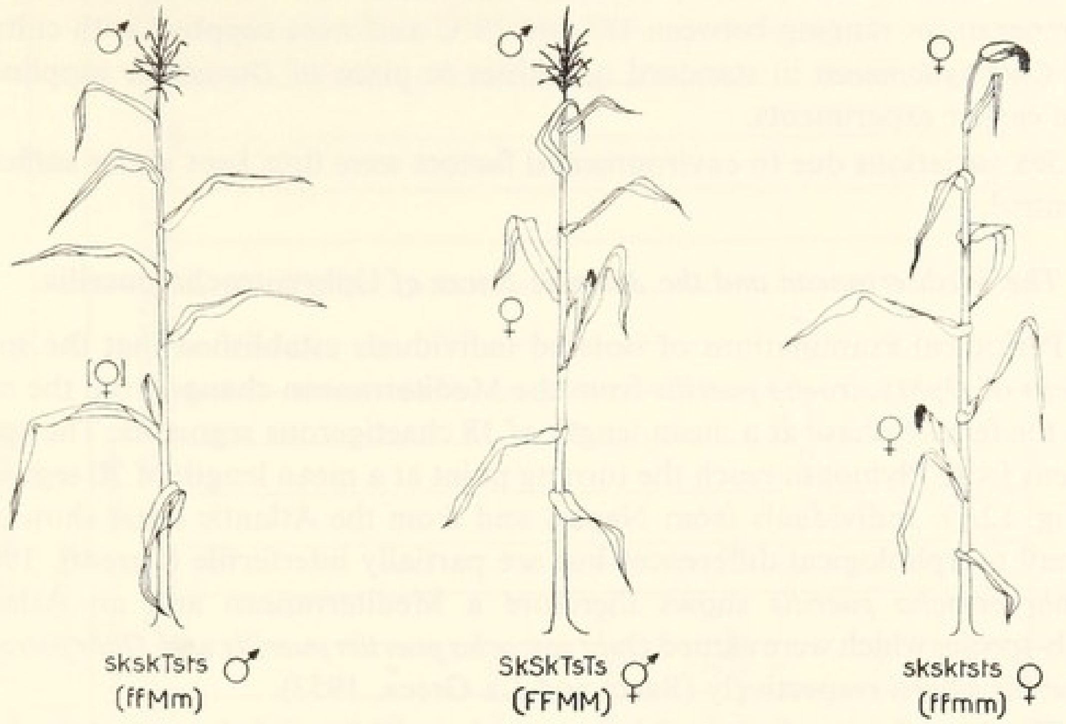


FIG. 8.2. Silkless (Sk) and tassel seed (ts) mutations produce dioecious plants by inhibiting the production of female and of male flowers respectively.

It is difficult to regard the experiments, that have developed a dioecious strain in maize, as valid models for the evolution of dioecy from monoecy. They suggest an evolution through inhibitory actions upon the genetic sex background and imply the coincidence of two opposed and complementary mutations with the formation of some isolating mechanism of the new dioecious individuals from a primitive monoecious population.

The action of the silkless and tassel seed genes shows, however, as already mentioned, a strong resemblance with the inhibitory effects of some regions of the *Melandrium* sex chromosomes. They correspond in a monoecious organism to such sex-modifying factors like the transformer gene of *Drosophila.*

The study of silkless and tassel seed has provided, therefore, valuable information about the possible sources of genetic variability among hermaphrodites.

The Multiple Sex Genotypes of *Ophryotrocha puerilis*

Selection experiments on *Ophryotrocha puerilis* (Bacci and co-workers, 1955–1961) provided some information upon the existence of multiple sex genotypes in unbalanced hermaphrodite animals.

Hartmann's experiments had shown a marked modifying influence of the environmental conditions and therefore it was necessary to keep the cultures in an optimal and possibly constant medium. The animals were kept at temperatures ranging between 18° and 19°C and were supplied with cultures of *Chlamydomonas* in standard quantities in place of *Dunaliella* supplied in the earlier experiments.

Sex variations due to environmental factors were thus kept under sufficient control.

1. The Mediterranean and the Atlantic Races of Ophryotrocha puerilis.

Periodical examinations of isolated individuals established that the specimens of *Ophryotrocha puerilis* from the Mediterranean change from the male to the female phase at a mean length of 18 chaetigerous segments. The specimens from Plymouth reach the turning point at a mean length of 20 segments (Fig. 12.1). Individuals from Naples and from the Atlantic coast show also small morphological differences but are partially interfertile (Parenti, 1961). *Ophryotrocha puerilis* shows therefore a Mediterranean and an Atlantic sub-species which were named *Ophryotrocha puerilis puerilis* and *Ophryotrocha puerilis siberti* respectively (Bacci and La Greca, 1953).

The hypothesis of a possible genetical variability of the sex phenotypes inside each population (Bacci, 1951d) thus found support in the existence of racial differences in sex expression.

2. Pure Males and Females through Selection of Hermaphroditic Strains

Strains of *Ophryotrocha* from Naples and from Plymouth were employed for selection experiments in the attempt of influencing the time at which sex reversal occurs (Bacci, 1955).

The first series of experiments lasted only four generations and the results were positive both for the anticipation of the female phase and for the prolongation of the male phase in the five strains that were investigated.

Individuals that never passed to the female phase, or began to show oocytes at the mean length of 25 segments were obtained at the fourth generation from one of the strains whose individuals passed to the female phase at a mean length of about 18 segments in the first generation. Selection against duration of the male phase in another strain produced on the other hand individuals that began to show oocytes at 11 segments in a generation whose individuals passed to the female phase at a mean length of only 13 segments.

The individuals that showed oocytes at 11 or 12 segments practically suppressed the male phase and were thus considered as pure females. The individuals that died at lengths between 20 and 26 chaetigerous segments without having produced any oocytes were likewise assimilated to pure males

Selection for the male and for the female phase

First appearance of oöcytes																																	
Gen.	P	N	M	Number of chaetigerous segments																													
				11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
0		148	18,79						5	15	43	45	26	12	1	1																	
1	20.20	122	20,56								8	13	35	40	22	3	1																
	17.17	145	17,98					2	12	36	53	22	18	2																			
2	23.23	127	22									4	10	29	37	37	6	4															
	17.17	103	16,80				3	10	23	38	26	3																					
3	23.23	72	22,76										1	13	18	17	16	7															
	15.15	170	16,44				2	29	54	63	21	1																					
4	25.26	98	22,97									4	5	14	20	14	19	13	5	3	1												
	14.14	133	16,70				2	11	40	54	23	3																					
5	28.31	6	24												2	–	1	2	1														
															and 5 pure ♂♂																		
	34.35	10	27,40												1	2	2	1	–	–	–	–	2	–	–	–	–	–	1	1			
																						and 2 pure ♂♂											
6	30.30	10	30,70										1	1	1	1	–	–	–	–	–	–	–	–	1	–	–	1	–	1	1	1	1
																						and 1 pure ♂											
	36.37	4	26,75															2	–	–	1	1											
																		and 6 pure ♂♂															

TABLE 3.

although they did not reach the full length of the Mediterranean sub-species which can reach 36 segments.

Thus, even if the identification of pure males appeared still doubtful in the first series of experiments, the possibility of the formation of prevalently male and female strains from an unbalanced hermaphrodite was well established in accordance with previous research on *Patella*.

The above experiments also showed that monogenous strains can possibly originate from an unbalanced hermaphrodite population.

A new series of experiments has been started on strains of *Ophryotrocha puerilis siberti* from Roscoff. More numerous individuals were examined in each generation and the conclusions reached with the first cycle of experiments (Bacci and Bortesi, 1959–1961) were confirmed and extended.

The 0 generation, which is the progeny of a single couple of individuals, passes from the male to the female phase at a mean length of 18·8 segments as shown in Table 3 where some of the results have been indicated. In generation 1 the sons of the specimens that were selected for maleness begin to show oocytes at mean values of 20·5 segments and the sons of couples selected for femaleness pass to the female phase at about 18 segments.

In generation 2, oocytes begin to appear at a mean length of about 22 segments in the groups selected for the prolongation of the male phase and the mean falls a little below 17 segments following selection for the female phase.

At generation 3, the minus individuals change sex phase at 16·4 segments, the plus individuals begin to show oocytes at a mean of 23 chaetigerous segments.

The selection for the female phase stops working at generation 4 where the progeny of individuals that have changed sex at 14 segments only shows oocytes at a mean of 16·7 segments. Results of tentative selection for the female sex are therefore not reported for the fifth and sixth generations.

The selection for the male phase appears on the contrary to have been effective in the next fifth and sixth generations where the number of the progeny of each couple has sharply dropped because individuals that change late to the female phase also produce very few ripe oocytes. Individuals that do not change sex, and are therefore pure males, appear frequently in the fifth and sixth generations where they reach between 40 to 47 segments (Fig. 8.3) without ever showing any oocytes.

Crosses between individuals of the second generation, which showed oocytes at 15 segments and individuals of the first generation which began to show oocytes at 23 segments produced a progeny that changed to the female phase at a mean of 18·69 segments as shown in the first row of Table 4.

This mean scarcely differs from the mean shown in the 0 generation (Table 3), a result which falls in line with those obtained in classic research work on polygenic determination. Individuals resulting from such a cross

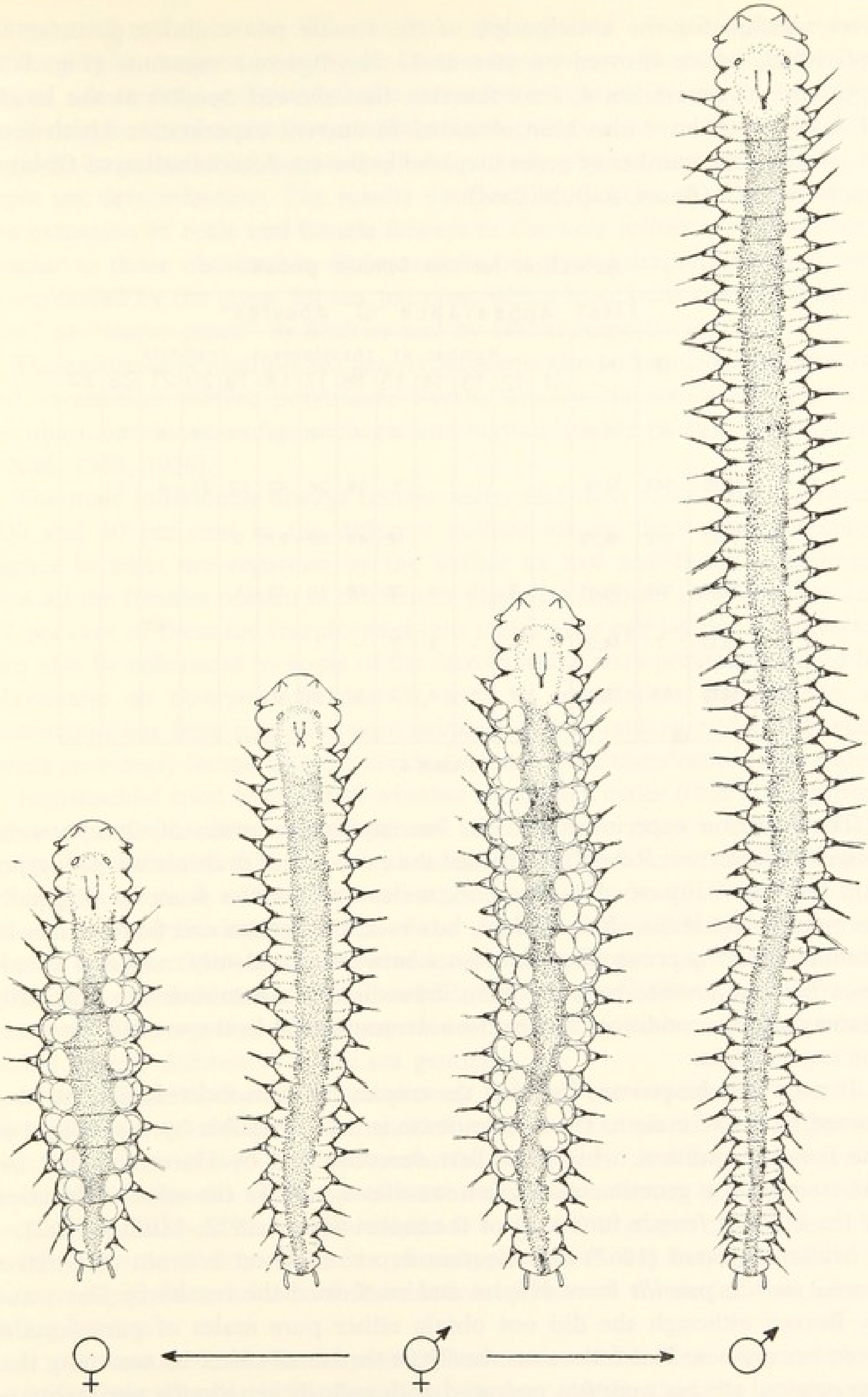
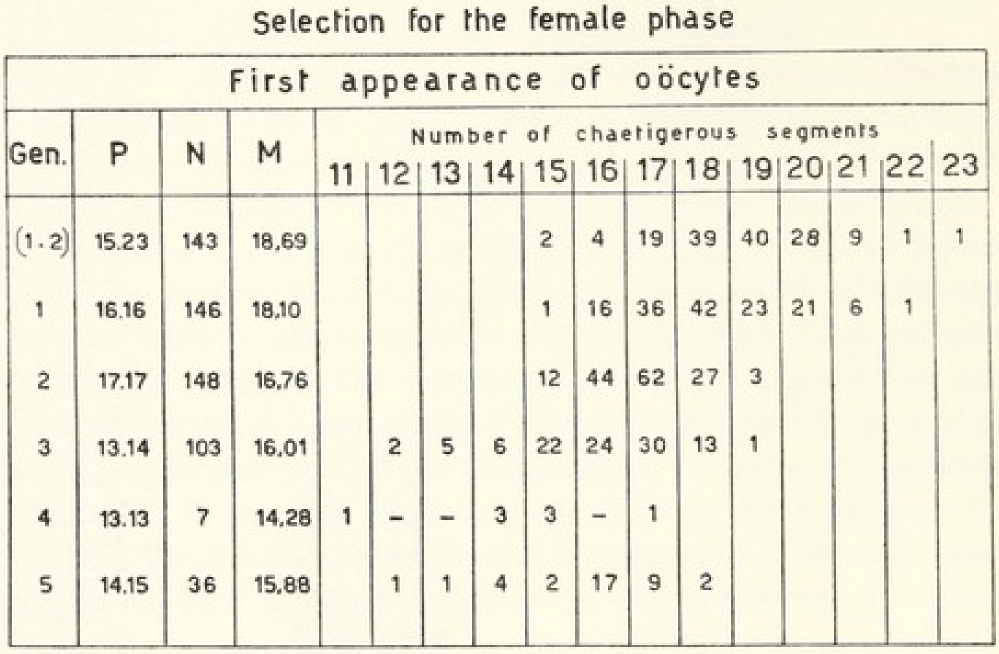


FIG. 8.3. Pure female and pure male individuals are obtained through selection from protandrous strains (centre) of *Ophryotrocha puerilis* (Bacci and Bortesi, 1961).

were selected for the anticipation of the female phase and a pure female individual, which showed oocytes at 11 chaetigerous segments (Fig. 8.3), appeared at generation 4. Pure females, that showed oocytes at the length of 8 segments, have also been obtained in current experiments which tend to establish the number of genes involved in the sex determination of *Ophryotrocha puerilis* (Bacci, unpublished).



Selection for the female phase

First appearance of oöcytes																
Gen.	P	N	M	Number of chaetigerous segments												
				11	12	13	14	15	16	17	18	19	20	21	22	23
(1.2)	15.23	143	18,69					2	4	19	39	40	28	9	1	1
1	16.16	146	18,10					1	16	36	42	23	21	6	1	
2	17.17	148	16,76					12	44	62	27	3				
3	13.14	103	16,01		2	5	6	22	24	30	13	1				
4	13.13	7	14,28	1	–	–	3	3	–	1						
5	14.15	36	15,88		1	1	4	2	17	9	2					

TABLE 4.

The selection experiments on the hermaphrodite strain of *Ophryotrocha puerilis siberti* from Roscoff confirmed the existence of multiple sex genotypes and the possibility of obtaining pure males and females from an originally hermaphrodite strain. It is evident, however, that males and females can be obtained only by preventing the crosses between prevalently male and female lines because crosses between them immediately re-establish the originally hermaphrodite condition, as has been demonstrated in the second course of the experiments.

It must also be pointed out that the crosses between individuals that have passed from the male to the female phase is made possible by the lability of the female condition, which was first demonstrated by Hartmann, but the influence of the genetic constitution manifests itself in the relative duration of the male or female functions of the mates (Bacci, 1952; Müller, 1962).

Müller repeated (1962) the selection experiments on a strain of *Ophryotrocha puerilis puerilis* from Naples and confirmed the results by Bacci and by Bortesi although she did not obtain either pure males of pure females from her crosses. This failure can easily be explained either by assuming that the original strains were not endowed with enough genetic sex variability or by assuming that the environmental conditions that were established in her cultures did not allow the differentiation of pure male or female phenotypes.

The results obtained on both sub-species of *Ophryotrocha puerilis* have thus solved some of the problems that were posed by the study of sex variability in hermaphrodites and have established the existence of multiple sex genotypes in a species which was thought to provide a typical example of phenotypic sex determination. The results obtained by Shifriss and by Galun on the extension of male and female flowers in *Cucumis sativus* are indeed very similar to those obtained in *Ophryotrocha* but their interpretation has been complicated by the genes for sex patterns, which have been named "qualitative" or "major genes" by Shifriss and by Galun respectively.

The existence of multiple sex genotypes seems also to have been demonstrated in another marine polychaete worm, *Grubea clavata*, which shows a peculiar combination of gonochoric and hermaphrodite phenotypes (Hauenschild, 1953, 1954).

The male individuals always remain males and their ratio ranges between 100 and 30 per cent in the different culture media, their ratio becoming higher in what are regarded by the author as bad nutritional conditions. Not all the females remain in the female phase throughout their life cycles as 37 per cent of them are transformed into males after egg laying. Sex reversal can also be influenced by some of the factors which were previously tested by Hartmann on *Ophryotrocha puerilis*, such as amputation, hunger, etc. A distinction has been made between primary and secondary males, the latter being previously female phase individuals which were transformed into males.

Hauenschild tried to establish whether secondary males (that is the protogynous hermaphrodite individuals) and primary males (that is the pure male individuals) showed different sex phenotypes, and he failed in demonstrating such differences in a first series of experiments. Hauenschild decided on a purely phenotypic determination of the sex expressions (1954) but later research on new *Grubea* strains—which were kept in suitable environmental conditions—demonstrated (Hauenschild, 1959) marked differences in the sex ratios of the progenies of primary and of secondary males and thus established genetic differences in the sex genotype.

It is difficult to decide, at the present stage of the experiments, whether *Grubea clavata* can actually be regarded as a sure example of sex polygamety like *Xiphophorus helleri* and *Ophryotrocha puerilis* or if it must be regarded a sex digametic species whose sex ratio can easily be altered by environment induced reversals of the sex phenotype at early or late stages of the life cycle.

The extreme lability of the sex phenotype—which is a frequent feature in cases of polyfactorial sex determination—leads us to prefer the former interpretation.

It was remarked, as a conclusion of both series of selection experiments in *Ophryotrocha* (Bacci, 1955; Bacci and Bortesi, 1961), that the progressive reduction of male or of female phase at each generation until pure male and female individuals appear, leads to the segregation of predominantly male or female strains—in short, of arrhenogenous or thelygenous strains—from hermaphrodite strains. The genes that influence the duration of the sex phases when in heterozygous condition are the same that produce high proportions of male and female individuals in the fourth or fifth generation of selection, that is they function as sex ratio genes when in homozygous condition. The demonstration that secondary males of *Grubea clavata* generate higher proportions of females than primary males adds further evidence for the identity of genes affecting sex differentiation and sex ratio among Polychaetes. The similarity in the sex-determining mechanism of *Ophryotrocha* and of *Xiphophorus* would thus be explained on the grounds of such twofold effect of multiple sex genes.

Environment and Sex Genotypes in Hermaphrodites and in Labile Gonochorists

A general discussion on hermaphrodite and sex labile species is now possible after the most significant observations and experiments have been summarized. The realization that sex determination is based, in many species, on mechanisms which result in sex polygamety led to a re-examination with new criteria of the series of observations which appeared to have been fully explained by the theory of phenotypic sex determination.

1. The Environmental Factors

The environment may exert a very strong influence on sex determination. The study of its effects is comparatively easy and this partially explains why a great deal of research has been carried on in this field and also why some authors were induced to attach primary importance to environmental factors in sex determination. It is impossible at present to give a satisfactory classification of such factors and therefore a descriptive enumeration only can be made.

Amputation may induce sex reversal from female to male phase in *Ophryotrocha puerilis* and Hartmann (1943) thought that such effects were due to rejuvenation until new experiments implied such effects to altered metabolism in unfavourable culture conditions (Bacci, 1951, 1952; Müller, 1962). The same results were obtained by Hauenschild in his *Grubea* research. Removal of gills increases the proportion of male phase individuals in *Ostrea gigas* (Amemya, 1929). It must also be recalled that castration induces sex reversal in such digametic animals as hens (Champy, 1939).

Results obtained by Hartmann in *Ophryotrocha* and by Hauenschild in *Grubea* showed that hunger may induce sex reversal from the female to the male phase and shortage of food also produces a high proportion of males in the copepod *Cyclops viridis* (Metzler, 1957). As the excision of gills affects nutrition in oysters, it appears that the prolongation of the male phase, which has been observed by Amemyia in *Ostrea gigas*, may actually be due to hunger.

Crowding also influences the succession of sexual phases in oysters by extending the male phase (Coe, 1932; Burkenroad, 1937). The percentage of male sex phenotypes is also increased by crowding in *Paramermis* (Caullery and Comas, 1928; Parenti, 1962) and in *Mytilicola* (Bacci *et al.*, 1958). It has not been established, however, whether sex is influenced in such instances by nutritional deficiencies, by specific hormone-like substances, by accumulation of waste products or by the joint influence of such factors.

The influence of the inorganic components of the surrounding medium was analysed by Herbst (1928–1940) in his experiments on *Bonellia* larvae. The addition of K^+ ions and the subtraction of Mg^+ ions from normal seawater causes, in Herbst's interpretation, a removal of water from the developing larvae and has thus a male determining influence. The elimination of K^+ increases the introduction of water in the larvae, favours a quicker development and their differentiation into female individuals. This interpretation has also been applied by Herbst to Hartmann's experiments on *Ophryotrocha*. Sex determination in general would be influenced through a regulation of the water content.

An interesting study on the effect of external agencies on the sexuality of *Tigriopus japonicus*, a marine copepod, was made by Takeda (1950). The copepod is strictly gonochoric and the influence of various substances is expressed by the altered sex ratios. It was found that di-iodotyrosine, which accelerates larval growth in *Tigriopus*, increases the percentage of males to 70 per cent. Chloretone, chloral hydrate, KCl and $KClO_3$, which on the contrary lessen the larval growth, considerably increase the percentage of female individuals. The stage when such influences are effective is nauplius IV and, in Takeda interpretation the substances influence the sex by inducing variations of the viscosity of the protoplasm. Subsequent researches by Egami (1951) on the same species investigated the action of other factors like temperature, $MgCl_2$, $MgSO_4$, NaCl, LiCl, etc. with substantially similar results which established that sex is not fixed until the last nauplius stages.

The influence of temperature on sex ratio in *Gammarus duebeni* and *Gammarus salinus* has also been investigated by Kinne (1952, 1953). In *Gammarus duebeni* temperatures below 5°C produce a high percentage of male individuals and temperatures above 6°C abruptly produce a high prevalence of females. Kinne's researches do not give information about the stage when sex is fixed. The researches by Metzler (1955, 1957) on the copepod *Cyclops viridis* have already been discussed in connexion with the *Talaeporia* case because sex in *Cyclops* is firmly determined at fertilization through a mechanism of female digamety.

An analysis of the influences of food components on sex ratio has also been carried on by v. Dehn (1950, 1955) in a series of researches on the cladoceran *Moina rectirostris* and completely phenotypical sex determination was assumed for this heterogonic species as for other species of cladocerans. New researches (Bacci *et al.*, 1961) on the maturation of the parthenogetic eggs permit, however, a different interpretation which will be discussed in Chapter 11.

The most interesting among the external sex determining factors appear to be the hormone-like substances whose existence has been first suggested in the experiments on *Bonellia*; and later in *Ophryotrocha* and *Ione* experiments. The recent observations by Müller that contact is necessary in *Ophryotrocha* casts, however, some doubt about the mode of action of such substances. A sex determining influence of the *Bonellia* type has also been suggested for *Mytilicola* and for *Paramermis*. Although such influences appear to be extremely important because they are active in the normal life cycle of the species almost nothing is known about the chemical nature of the substances involved, about their specificity and about their mode of action. Much more investigation is needed in this field.

It appears meaningless to try to evaluate the importance of environmental influences on sex determination. It is nevertheless necessary to point out once more that even in animals where sex digamety has been established both through cytological and genetical methods, sex inversion and alterations of sex ratios have been obtained by means comparable to those mentioned above. For this reason it is not justified to draw any definite conclusion regarding the sex genotype from experiments that show the influence of the environment on the individual sex phenotype leaving out of consideration sex variation and its inheritance in the progeny (Bacci,1950a). In other words the validity of the FFMM formula cannot be proved through the study of environmental influences alone.

2. *The Sex Genotypes*

The discovery of the high sex variability in *Patella* led to the realization of two important facts.

(a) Some hermaphrodite species are composed of a majority of hermaphrodite and of a few pure male and pure female individuals and it was assumed that from such species both purely (balanced) hermaphrodite species and gonochoric species (Bacci, 1947a, c) can be developed.

(b) Some gonochoric species that show labile gonochorism can be assimilated with unbalanced hermaphrodite species from the point of view of sex variability (Bacci, 1947b).

Such facts led Montalenti and the author of the present book to advance a general theory to explain both the sex gradations of unbalanced hermaphrodite species and of *Bonellia* type labile gonochorists (Bacci, 1949b; Montalenti, 1950; Bacci, 1950; Montalenti and Bacci, 1951).

Table 5 (from Bacci, 1949a, 1950a) shows how the theory can explain the appearance of the different phenotypes in unbalanced hermaphrodite species, as exemplified by *Patella* and by *Crepidula* and by *Bonellia* among unbalanced labile gonochorists.

It was assumed that sex is determined in *Patella* by four couples of factors. The duration of either sex phase results prevalently from the Mendelian segregation of multiple sex genes, whose action is additive. The most numerous class of individuals is thus represented by hermaphrodites where the existence of an equal number of genes for the male and for the female sex produces equivalent expressions of maleness and femaleness in the same individual. The two classes at each side represent individuals whose genotypes contain five or six male genes and three or two female genes or *vice versa*. Hermaphrodite conditions showing a stronger or weaker male tendencies are thus obtained. They are expressed in *Patella* by the fact that the heterozygotes of the four predominantly male or female classes will change sex phase at later or at earlier stages than the hermaphrodites showing sex phases of equal durations. The co-existence of seven male factors and one female factor in the same zygote will form primary males as it is supposed that the sex balance will ensure the stability of the male sex phase. A similar interpretation is given for the combination showing a prevalence of the female sex genes. Also homozygotes, if they do exist at all, will form either pure male or pure female phenotypes.

A statistical investigation on 9588 *Patella*, which were collected in September soon after the period of maximum sex reversal was over, showed that the percentages of individuals that change from the male to female phase are distributed in accordance with the above hypothesis (Fig. 8.4).

It was pointed out, however, that the multiple factor hypothesis provides only an orientative interpretation of sex variability in *Patella* and that it does not exclude a modifying influence of the environment on sex variability (Bacci, 1949b).

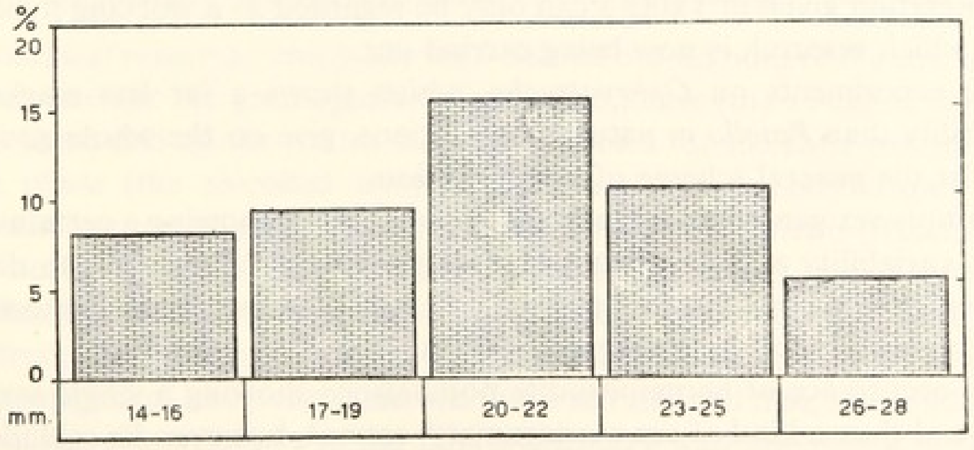


FIG. 8.4. Percentages of individuals that undergo sex reversal at different size classes in the unbalanced hermaphrodite *Patella coerulea* (Bacci, 1949b).

The sex phenotypes of *Crepidula plana* have been exemplified on the basis of Coe's research.

Observations by Wilccynski (1959) on a supposed sexual dimorphism of the *Crepidula* eggs may be considered as indicating a sex polarity rather than a sharp division into two sex classes, as occurring in digametic species. Such interesting cases of dimorphism may, however, have nothing to do with sex differentiation.

In *Bonellia viridis* the different classes of individuals are based on the different degrees of sex lability of the larvae which are assumed to correspond to different reaction norms of the sex genotypes: the larvae that become males even in the absence of an adult female (the Spätmännchen) are assimilated to the pure males and the larvae that will not adhere to the proboscis of the ripe female are assimilated to the pure females of the unbalanced hermaphrodite populations. The data that are now available in the *Bonellia* literature do not permit us to evaluate the proportions of the different classes of sex phenotypes and it is only known that Spätmännchen, and pure female larvae represent a small minority of the population in comparison to the sex labile larvae.

A similar interpretation can also be applied to the *Paramermis* case (Bacci, 1955) where racial differences in the reaction to the environmental conditions have been demonstrated (Parenti, 1962a).

It is evident that the above interpretation of the sex determination in labile unbalanced gonochorists implies also the existence of a delicate switch mechanism of the type suggested by Kosswig for *Xiphophorus helleri* and for other cyprinodont fishes.

It has been shown in Chapter 3 that the phenomenon of relative sexuality (which is presented by haplophase individuals) is susceptible of a similar interpretation.

Much work must be done indeed before we will obtain a fully satisfactory interpretation of the genetical background in labile gonochorism and the interpretation given in Table 5 can only be regarded as a working hypothesis upon which research is now being carried out.

The experiments on *Ophryotrocha*, which shows a far less marked sex variability than *Patella* in natural populations, give on the whole good support for the general scheme of interpretation.

Multiple sex genotypes are present in populations showing a certain degree of sex variability and they produce multiple kinds of gametes with different degrees of sex potency. Sex polygamety has therefore been demonstrated in hermaphrodite as in monogenic species.

The occurrence of hermaphrodite populations showing a single sex genotype (and thus a kind of sex monogamety) cannot, however, be excluded and certain balanced hermaphrodite species like *Calyptraea chinensis*, as well as labile gonochorists of the *Ione* type may indeed be characterized by an extreme

GENOTYPES	% FREQ.	PHENOTYPES IN:		
		PATELLA COERULEA	CREPIDULA PLANA	BONELLIA VIRIDIS
8 dom. 0 rec. 7 dom. 1 rec.	0.4 3.1	Pure ♂	Pure ♂ (associated) with indefinite length of the male phase	Pure ♂ (Spätmännchen)
6 dom. 2 rec. 5 dom. 3 rec.	10.9 21.9	⚥ with long ♂ phase	⚥ with long ♂ phase	Individuals which are easily masculinized
4 dom. 4 rec.	27.4	⚥ with equal length of ♂ and ♀ phases	⚥ with equal length of ♂ and ♀ phases	Individuals which are equally easily masculinized or feminized
3 dom. 5 rec. 2 dom. 6 rec.	21.9 10.9	⚥ with short ♂ phase	⚥ with short ♂ phase	Individuals which are easily feminized
1 dom. 7 rec. 0 dom. 8 rec.	3.1 0.4	Pure ♀	Pure ♀ (isolated)	Pure ♀ (larvae which will not adhere to the proboscis)

TABLE 5.

uniformity of the sex genotype although the evolutionary possibilities of such hypothetical condition would be extremely reduced.

Genetic mechanisms that might determine such extreme expression of balanced hermaphroditism or labile gonochorism can only be supposed at the present stage of research. One of them may be represented by the fixation of the male and female sex genes through the suppression of the crossing-over. The few cytological researches that were expressly carried out on chromosomes of hermaphrodite species, like *Lepas* and *Scalpellum* (Witschi, 1935; Callan, 1941) do not give any useful information about such possibility. They might be resumed with some hope now that the exploration of sex phenotypes in hermaphrodites is being systematically carried out.

3. *Genic sex balance in* Ophryotrocha puerilis, *a homeostatic mechanism*

Cytological research, which has been carried out on both of the strains that were selected for the prolongation of the male phase (the so-called arrhenogenous lines) and on the strains that were selected for the anticipation of the female phase (the so-called thelygenous lines), has recently shown some important features of genic sex balance in *Ophryotrocha puerilis* (Bacci, 1965).

The normal gonadal differentiation had to be previously reinvestigated on individuals that were either collected from natural populations or were taken from freshly established cultures. It was shown that the last protogonial division originates one oocyte and one nurse cell: the cell that, as a result of the division, remains adjacent to the intersegmentary wall differentiates into an oocyte and the one that projects into the coelomic cavity develops into a nurse cell (Parenti, 1962; Zunarelli, 1963).

Individuals from the 4th generation of arrhenogenous lines showed marked deviations from the normal development of the female phase gonads. They consist mainly in a slight differentiation of nurse cells and oocytes both between themselves and with regard to undifferentiated germ cells outside the gonad. Individuals from the 4th and 5th generations of thelygenous lines show on the contrary a marked vacuolization of the cytoplasm of primary and secondary spermatocytes and a peculiar stickiness of the spermatids. Such disturbances of the male and female gonadal differentiation—which are clearly due to the selection against the male and the female factors—led also in a few individuals to the unchecked proliferation of undifferentiated germ cells, which may fill out the coelomic cavities of the worms.

Alterations of gonadal differentiation took place on the other hand also during the sex phases that were favoured by selection. Pycnotic nuclei and sticky spermatids appeared, namely during the male phase in individuals belonging to arrhenogenous lines, and dwarf nurse cells were observed in the 4th or 5th generation of thelygenous lines. Altered spatial relationships between nurse cells and oocytes were also evident in such individuals.

A significant conclusion concerning sex balance can be deduced from such results. They show that prolonged selection for either sex phase alters the balance among sex factors in such a way as to alter the expression of both sexes. Selection affects in other words the sex phase against which selection is carried on and also the sex phase that is apparently favoured by it.

It has been shown in Chapter 5 that given thresholds of sex balance must be reached in *Drosophila melanogaster*, and presumably in all other sex digametic organisms, in order to obtain normal male or female phenotypes. The present work on the hermaphrodite *Ophryotrocha* shows that the normal expression of the male and of the female phase can only be obtained within given limits of balance between male and female determining factors. Such limits appear, however, to be not sharply defined because sex balance is not conditioned in *Ophryotrocha* by the presence of major sex genes or gene blocks that are located in the sex chromosomes.

The work on sex balance established also the principle of sex bipotency in unisexual animals. It seems meaningless to extend such principle to hermaphrodite organisms but the results on *Ophryotrocha puerilis* point to a new development because the factors of the complementary sex appear to be necessary for the normal expression of either sex. Sex bipotency can thus be interpreted as the result of the necessary co-operation between both sex factors in order to express either male or female characters.

The above mechanisms of sex balance also explain why pure male or female individuals of *Ophryotrocha puerilis* are not found in natural populations. It indicated that pure female individuals, which were mentioned in an early paper (Bacci, 1951c), actually belong to a different species that was later named *Ophryotrocha labronica* (La Greca and Bacci, 1962) and that so-called pure males did not reach the sizes which are obtained in laboratory experiments.

It is now clear that the above mechanism of sex balance reduces the fitness of the sex homozygotes which can be obtained in the sheltered laboratory conditions but fail to appear in nature. The gradually decreasing number of the progeny in the generations of arrhenogenous and thelygenous lines is also evident in Tables 3 and 4 and it can now be interpreted on the basis of the extensive damages suffered by sexual cells as a result of selection.

In this way sex homeostasis in natural populations of *Ophryotrocha puerilis* is ensured by its very mechanism of sex balance, despite polygenic sex determination.

It is still to be seen which is the mechanism of sex balance in such unbalanced hermaphrodite populations like *Patella coerulea* or *Asterina gibbosa* from Lake Fusaro.

CHAPTER 9

SEX GENOTYPE AND SEX PHENOTYPE

THE relationship existing between sex genes and sex characters through the chain of processes which take place between the time when the sex genotype is established in the haploid spore or in the zygote and sex phenotypes are expressed in the adult individuals has only been fragmentarily understood in a few organisms. The little we know is nevertheless sufficient to show the great diversity of the mechanisms by which the sexual differentiation is obtained in various groups of organisms. Nothing is known about the most immediate effects of sex genes both because the operational genetic units for sex differentiation are unknown to us and because primary gene actions can only be investigated in a few organisms. For this reason a unifying principle in understanding the sex differentiating processes is still lacking.

Sex digamety leads to the differentiation of unisexual individuals and yet this uniform result is obtained through an unpredictable versatility of developmental patterns in the different phyla. The differentiation of male secondary sex characters is determined in amphibians by the integrated actions of testicular and hypophysian hormones (see p. 191), but in malacostracous crustaceans it is determined by hormones from the androgenic gland (see p. 196) which is totally absent in vertebrates. No trace of the cortical and medullary territories, which play an important role in the gonadal differentiation of many vertebrates, has ever been found in crustaceans. Similar examples could easily be multiplied, but the analysis of the present chapter will be restricted to the Amphibia and Crustacea.

Recent research leads to the conclusion that variability due to sex polygamety is expressed quite differently in different species. It has been demonstrated that polyfactorial sex determination results in differences in the sex ratios of the progeny in cyprinodont fishes (Kosswig), in isopods (De Lattin) and in copepods (Battaglia) and a similar gene mechanism is responsible for different durations of sex phases in unbalanced hermaphroditism (Bacci). It is also highly probable (Bacci) that some kind of polyfactorial determination is responsible for the varying degrees of sex lability in *Bonellia* and even for the phenomena of relative sexuality, as shown in other chapters. Different sex conditions, and not only differences in the sex differentiating mechanisms, are therefore observed among species that follow the same general patterns of sex determination. How this may come about can only be guessed through the scanty information available and provides an important matter for future investigation.

It is therefore important for the biologist interested in the problem of sex determination to realize that the most varied expressions of sexuality may conceal similar mechanism of gene inheritance and that, conversely, quite similar sex phenotypes may be obtained through different types of gene inheritance. This is not surprising because sex determination is concerned primarily with genetic, and sex differentiation essentially with embryological mechanisms.

Our most important source of information about sex differentiation comes from the study of diploid individuals which, curiously enough, imprint the sexual characters upon the haploid gametes through the inductive action of somatic elements, as it was demonstrated by research on vertebrate development.

Research on the gamete formation in haploid organisms has not fulfilled the promise given by early research on *Chlamydomonas* although the work on the flagellate symbionts of *Chryptocercus* and the work on haploid fungi are now yielding extremely interesting results.

The following examples are taken from amphibians which provide a model for sex differentiation in vertebrates and from crustaceans where new interesting studies have recently been carried out.

Sex Differentiation in Amphibians

1. Germ Line and Gonad Differentiation in Amphibians

Early differentiation of germ line was established in amphibians through a combination of cytological observations and embryological experiments.

Primitive germ cells from the dorsal entoderm were first recognized by Allen (1907) in *Rana pipiens* and by Beccari (1920) in *Bufo*. An earlier localization of the primitive germinal cells was demonstrated in *Discoglossus pictus* by Monroy (1939) who obtained complete sterility after removal of the whole or the ventral half of the entoderm in neurula stage, contrary to the effect of the removal of the dorsal entoderm.

Bonoure (1939) was able to trace a primary localization of special germinal cytoplasm at the vegetative pole of the fertilized egg in *Rana temporaria*. The pregastrulation movements displace the germinal cytoplasm to the central blastomeres and at the beginning of gastrulation the presumptive germ cells are characterized by a perinuclear cytoplasmic area that can be demonstrated by means of special staining methods. The primitive germ cells are then displaced to the caudal entoderm, from where they move to the germ ridges passing through the dorsal entoderm. A similar origin of primitive entodermal germ cells takes place also in birds where Dantschakoff (1941) demonstrated their migration from the germinal crescent, located in an extra-embryonal area, to the genital ridges.

Radiation with ultraviolet rays of the vegetative pole of the egg of *Rana temporaria* produces almost complete sterility of the gonads (Bounoure, 1937) and this result has been recently confirmed by Padoa (1963) who irradiated unsegmented fertilized eggs of *Rana esculenta* and obtained tadpoles with the gonads entirely devoid of gonocytes. It is interesting to notice that the same treatment has been found to give no result whatsoever when applied to early blastulae.

Morphological analysis as well as experimental evidence thus indicate an entodermal origin of the germ cells in anuran amphibians. Research on urodeles gave rather contradictory results as a mesodermal and an entodermal origin of the germ cells were assumed by Humphrey (1925, 1929) and by Bonoure (1925) respectively. The work of Neeuwkoop showed the presence of an activating centre for the germ cell differentiation in the entoderm of some species of urodeles because any obstacle to the contact between entodermal and mesodermal territories determines either the decrease in number or the absence of germinal elements (1950). Such results seem to lend support to observations by Ranzoli (1959) who has found numerous cellular elements of apparently germinal type in the entoderm of young tadpoles of *Triturus cristatus.* Such elements migrate in later stages into the mesoderm near the pronephric ducts which some authors regard as the only precocious seat of germinal elements in urodeles. If Ranzoli's observations are confirmed the origin of germinal elements in urodela will not appear as exceptional among vertebrates. It is worth mentioning that Blackler has found a germinal plasma, which resulted to be very rich in RNA, in the eggs of *Xenopus laevis* and of *Bufo bufo* but not in the eggs of *Rana esculenta* where Padoa's experiments have demonstrated a germinal localization.

Some discussion is still going on regarding gonad differentiation. In amphibians, as in all vertebrates, gonads differentiate at first as two elongated ridges that are situated on the roof of the abdominal cavity and are composed of elements from the mesenchyme and of primordial germ cells or protogonia which are covered by epithelial coelomatic cells. A primordial genital cavity originates in many species after the genital ridge has been formed and cellular strings penetrate the gonads through the ilar region until they jut out into the genital primordial cavity.

In this way two territories come to be distinguished in the still sexually undifferentiated gonad: the cortex which is prevalently composed of protogonia and of a few mesenchymal cells and is covered by the coelomic epithelium, the medulla which forms the central axis and is initially composed by mesenchymal cells (Fig. 9.1).

In the ensuing processes the two territories undergo a different development and male or female individuals develop depending whether the cortical or the medullar development prevails: the cortex has been regarded a male and the medulla a female inductor (Witschi, 1914).

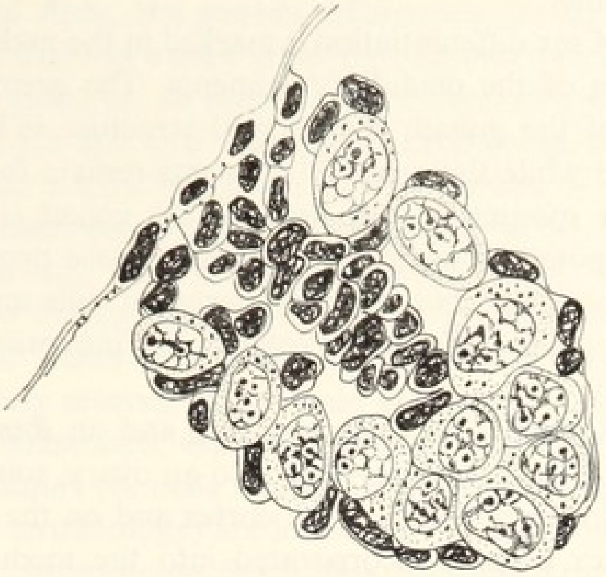


FIG. 9.1. Cortex and medulla in a gonad of *Rana agilis* which shows resting oogonia (from Vannini, 1938).

The separation of the embryonal gonad into two somatic territories has been demonstrated also among the amniotes (Dantschakoff, 1941) and among selachians (Chieffi, 1949) and it must be considered a basic feature of sex differentiation in vertebrates with the exception of teleosts (D'Ancona, 1949).

The double system of territories expresses therefore in most vertebrates their genetic sex bipotency. In the original Witschi's interpretation (1914, 1929) the cortex derived from the coelomatic wall and the medulla from the mesonephric blastema. According to Vannini (1941–1950) the medulla originates from the same blastema from which the inter-renal organ originates. Cytochemical researches by Chieffi (1955), that enabled one to trace the elements of the inter-renal blastema up to the very earliest stages, gave support to the view of a close relationship existing between the development of the somatic component of the genital organs and that of the inter-renal organ. Further research by Vannini and Sabbadin (1954) indicated that not only the medulla but also a part of the cortex are developed by the blastema of the inter-renal organ.

Researches by Witschi (1952–1953) and by Padoa (1959) tended on the other hand to exclude the separate origin of the mesonephric and of the inter-renal blastemas, as was early assumed by Vannini, and the conclusion was reached that both the inter-renal organ and the somatic elements of the genital crest originate from a single primordial blastema although at different times.

The above studies showed that the inter-renal organ and the somatic cortical and medullary tissues of the gonads have a common embryological origin. This appears, as remarked by Vannini (1954) to agree with the chemical and physiological similarity of the steroid hormones that are secreted in the adults.

The beginning of sex differentiation is marked in the male genotypes by the active proliferation of the medullary elements. The germinal cells migrate into the interior of the gonad, the cortical structure is lost and testicular tubules are formed while the germinal elements remain for a long period at the protogonial or spermatogonial stage. If the gonad is going to develop into an ovary the potential testicular medullary tissue proliferates very little and becomes excavated at its centre; the germinal cells undergo a period of active multiplication and evolve into ovogonia and into primary oocytes which grow rather rapidly.

Researches by Sabbadin on *Rana esculenta* and on *Rana dalmatina* (1951 a, b) showed that, if the gonad develops into an ovary, some early medullary elements become incorporated into the cortex and on the contrary, parts of the primitive cortex become incorporated into the medulla if a testicular development takes place. Thus genetic sex factors appear to operate on somatic territories that are not irreversibly determined from the beginning.

Although Vannini and Sabbadin (1954) lay stress upon the fact that the distinction between cortex and medulla is not definitely established until late stages, Witschi's original interpretation of the cortex and of the medulla as inductors of female and male differentiation respectively retains its fundamental validity. It appears highly probable that the inductive element of the cortex are the follicle cells and that the interstitial cells are the inductive elements of the medulla. The inductive substances that are presumably released in the two territories were named cortexin and medullarin (Witschi, 1931) and the two inductors act as a pair of antagonists, each tending to suppress the other (Witschi, 1936). Humphrey's experiments on *Amblystoma* (1938) gave indications regarding the relationship between sex inductors and sex genotype in that they demonstrated that the power of inductors is under the control of the individual genotype. A lateral portion of the lateral mesoderm was transferred from young tadpoles to the same positions in other tadpoles at the same stages of development. Both tadpoles were later examined at a stage of development that allowed recognition of the sex of the gonads. It was found that the transplanted mesoderm developed a male or a female gonad according to the sex shown by the corresponding gonad of the donor. The sex of the recipient organism initially appeared to have no influence on the sex of the transplanted gonad. Thus the early gonadal differentiation is surely genotypically controlled at early stages of the development.

2. *Differentiated and Undifferentiated Races of* Rana *and the F and M Reaction Curve*

Research on sex variability in various species of *Rana* gave very important information both on sex determination and on the action of sex genes in amphibians.

In some races of *Rana*, the gonads differentiate directly into testes in the half of individuals that has a male genotype and into ovaries in the other half that has a female genotype. In other races of *Rana* all the individuals differentiate at first as females and only after metamorphosis, the half of the individuals that has a male genotype, undergo a sort of sex reversal with a transient female phase and eventually show gonads with a testicular structure. The races showing early male differentiation are called "differentiated" and those showing late differentiation are called "undifferentiated" races. Also "semidifferentiated" races exist which show an early ovarian differentiation but undergo an early reversal of the male genotypes.

It has been demonstrated, especially through the work of Witschi (1923, 1929), that male factors (or male realizators as they have been called according to Wettstein's terminology) are located in the autosomes, female factors especially in the X chromosome. Sex is thus determined through a mechanism of genic balance similar to that observed in *Drosophila* and the differences between the sex races have been explained as in *Lymantria* on the basis of differences in sex valences, with a "minor" female factor located in the Y chromosome. The Y of the undifferentiated races is less different from X than is the Y of the differentiated race. Contrary to the *Lymantria* case male digamety has nevertheless been demonstrated in the races of *Rana* investigated so far although no heteromorphic sex chromosomes have been surely demonstrated.

A valuable addition to the study of sex differentiation in amphibians was later made by Goldschmidt (1931) who developed an interpretation of the transient period of intersexuality in the genetic males of the undifferentiated races from a general theory of sex reversal. The male and female factors, which are now considered in their complex, are supposed to develop two independent chains of reactions that lead to the formation of male and female differentiating substances which are produced in increasing concentrations during the development of the individuals. Variations in the relative speed of such male and female reactions determine the passage from one to the other sex condition at a given moment which is called the turning point and was given much importance in the earliest Goldschmidt interpretations of the various degrees of intersexuality in *Lymantria*.

The production of female differentiating substances overcomes the production of male differentiating substances during the whole life cycle in female genotypes of both differentiated and undifferentiated races of *Rana*. The production of male inducing substances is on the contrary overcome by that of the female differentiating substances during the earlier period of the development in the male genotypes of the undifferentiated races. The turning point is reached at metamorphosis and the male development takes place in coincidence with accelerated production of masculinizing substances.

In a diagram by Padoa (1950) the fundamental principles of Goldschmidt's theory have been expressed by separating the three areas of female (below), intersexual (in the middle) and male differentiation with two distinct curves for males from differentiated and undifferentiated races and a single curve for the females. The abrupt turning point of the former Goldschmidt's scheme is replaced by the transition zone that indicates the transient intersexual period of the male genotypes (Fig. 9.2). Padoa's experiments (1947), which will be discussed later, demonstrated that the above explanation does not simply give a formal representation of morphological processes but corresponds to differences in the action of sex differentiating substances that can be experimentally demonstrated in amphibians, although it was not so in Lepidoptera (Seiler, 1946, 1949). A discussion of some basic experiments will throw some light upon the nature of such factors.

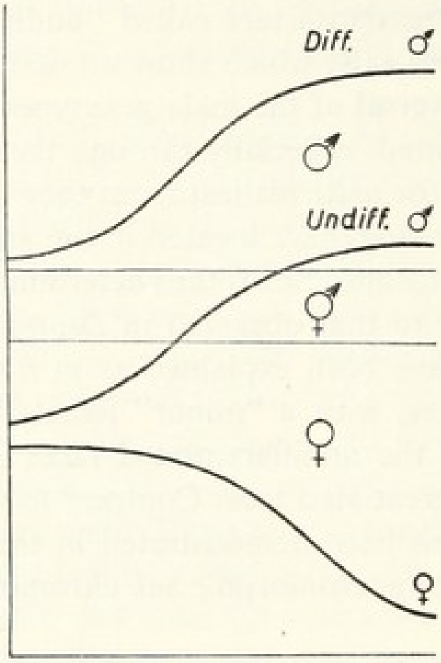


FIG. 9.2. The reactions determining male and female sex in differentiated and undifferentiated races of *Rana* according to Goldschmidt's and Witschi's theory (Padoa, 1950).

3. *The Somatic Induction of Sexuality in Germ Cells and the Impact of Environmental Factors*

The already mentioned Humphrey's experiments in *Amblystoma* (1938) demonstrated that the germ cells of the recipient tadpoles develop as male or as female gametes according to the sex of the donor tadpole under the influence of the transplanted mesodermal fragment into which they migrate. In other words they may develop as female gametes although they belong to a male individual and they carry XY chromosomes and conversely they may develop into sperms although they may have an XX constitution. It was clearly demonstrated by this experiment that the sex differentiation of the germ cells is determined in amphibians through the influences of the surrounding somatic tissues, a result that definitely confirmed previous conclusions by Witschi on the inductive activities of the two gonadal territories of amphibians.

This somatic induction of sex in germ cells also explains the possibility of sex reversal obtained in amphibians through a series of non-genic influences.

Over-ripeness of the egg produces in *Rana esculenta* the complete reversal of the female genotypes as shown by the experiments of Kuschakewitch (1923) who kept the eggs in the oviducts of the females for 89 hr and then inseminated them artificially after surgical extraction. From normal eggs were obtained 58 male and 53 female tadpoles, from the over-ripe eggs 299 female and 1 intersex only. Six per cent mortality was observed in the normal culture and only 4 per cent in the culture of tadpoles from over-ripe eggs.

Witschi confirmed (1924) Kuschakewitch's results and imputed the reversal to cytoplasmic alterations that in later development affect the medulla more than they affect the cortex. Successive work of Beetschen (1957) on over-ripe salamander eggs pointed to extensive irregularity in chromosome distribution at blastula stage but the relationship between such irregularities and disgenesis of the sex gland is still unclear.

Also an abnormally high temperature favours gonad differentiation into testes and a low temperature favours differentiation into ovaries as shown by Witschi in *Rana temporaria* and in *Rana sylvatica.* In case of development of male genotypes to female genotypes a persistence and a hypertrophy of the cortex takes place when the medullar development is prevented.

The reverse happens in female genotypes kept at high temperatures (Witschi, 1929). Similar results were obtained in *Bufo* (Piquet, 1930) and in salamanders (Uchida, 1937).

It has been mentioned already that sex reversal as a consequence of castration takes place in *Bufo* where the removal of the testes induces the hypertrophy of the Bidder's organs which give origin to functional ovaries.

Experiments on over-ripeness, high or low temperature and castration provide little information regarding the type of sex differentiating factors operating in normal development although the sex reversal represents an added proof of the genetic bipotency of both sexes and of the rather labile determination of sex in some amphibian species where sex chromosomes are but slightly differentiated.

Experiments on parabiosis which were initiated by Burns (1925) and were particularly developed by Witschi (1927) both on urodeles and on anurans provided some information regarding the sex differentiating substances. They indicate a different diffusibility in the various species that were investigated or, at least, they showed that different reactions to their influence take place in different species.

It is possible to unite two individuals at the tailbud stage just before the muscular activity begins and the fusion that takes place between the two embryos allows a circulatory exchange during the ensuing developmental stages. Embryos may be grafted laterally or head to tail. The first sexual differentiation of the embryos is normal both when homosexual and heterosexual pairs are formed. The subsequent gonad development, however, takes quite different directions in heterosexual pairs of salamanders, of frogs and of toads (Fig. 9.3).

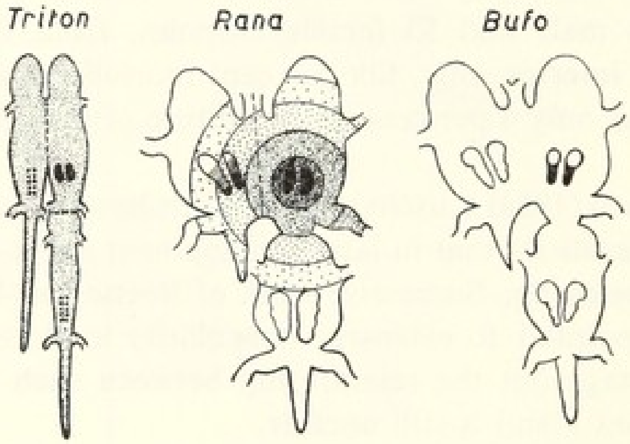


FIG. 9.3. Diagram illustrating the influence of the male gonad in parabiotic combinations of amphibians. Shading indicates the diffusion of the hormone-like substance which influences the differentiation of the heterologous gonad (Witschi, 1949).

The testes of urodeles develop normally as a rule but the ovaries of the partner having a female genotype undergo a strong involutive process and are reduced to very thin crests with very few, if any, germinal cells. This happens both in lateral and in head to tail pairs. The ovary exerts a feminizing effect upon the gonad of the male genotype only in heterospecific twins where the female belongs to the bigger sized species (Burns, 1935) or in twins where the female partner is diploid and the male is haploid (Gallien, 1963).

In the case of *Rana* a partial inversion takes place only in the female gonad that is nearest to the gonads of the male partner and no effect is observed in the more distant gonads.

In *Bufo* no interaction whatever can be observed between heterologous gonads. The sterilizing effect observed in the female genotypes of *Triton* and of *Amblystoma* recalls the so called "freemartin" effect which takes place in the foetal life of cattle and which was thoroughly investigated by Lillie.

In heterosexual cattle twins undergoing gestation in the same uterus, the female co-twin is often modified in the male direction and is sterile. It is named a freemartin. The concept that a male mammalian gonad is able to influence the differentiation of another gonad at a distance, was derived for the first time from the detailed study of such phenomenon. The experimental work on amphibians gave a general application to Lillie's interpretation.

In Witschi's conclusions regarding amphibian parabiotic twins (1934) much importance has been given to the distance between the gonad that produces the inhibiting substances and the gonad which undergoes its influence. The fact that in urodeles the inhibitory action of the inductors spreads over the whole twin system through the blood stream led Witschi to consider such substances as hormone-like. In toads, where such action is narrowly confined, and in frogs, where they seem to have a wider range, they are supposed to spread through the tissues. Although some criticism has been made concerning Witschi's conclusions regarding the difference in diffusibility of the sex inductors (Ponse, 1949), they posed with clarity the problem of the nature of the sex inducing substances at different stages of development and in different amphibian species. This problem has been more recently approached through the study of hormone action.

4. Hormone Action and Gonadal Differentiation

Treatment of thirty-six tadpoles from a differentiated race of *Rana esculenta* with a follicular hormone dissolved in water produced total masculinization of the individuals (Padoa, 1936), a result in striking contrast with previous results in chicks where this hormone demonstrated a clear female differentiating action (Willier and Gallagher, 1935). Thus the first data on sex reversal by hormone action in amphibians revealed a paradoxal effect that, although it was confirmed by Padoa (1938) and by Gallien (1940), represented an intriguing puzzle until Padoa (1942) showed that the addition of dihydrofolliculine to the culture water has different effects according to the doses. Small concentrations of the hormone in the water have totally feminizing effect, high concentrations (corresponding to those used in the earlier experiments) gave the paradoxal masculinizing effect. This work also demonstrated that the administration of steroid hormones through the culture water ensures an accurate dosage and permits a continuous treatment through the easy change of the water.

Sex reversals obtained with injections of oil-dissolved steroid hormones gave normal results in the sense that testicular hormones produced the inversions of the female and ovarian hormones of the male genotypes (Gallien, 1937–38; Burns, 1938) but the inversions were not always complete although high doses of crystalline hormone were employed. This demonstrates that, although high quantities of hormone are introduced with the injection method, hormone concentrations never reach a high level in the organism.

The method of the addition of steroid hormones to the culture water gave a quantitative value to the curves derived from the Goldschmidt's interpretation regarding the chains of reactions of the male or female determining substances (Padoa, 1947). The experiments were made on tadpoles of an undifferentiated race of *Rana dalmatina* and lasted from the twentieth to the forty-sixth day of age. Water with the addition of testosterone at concentrations of 5, 10, 20, 40, 80, 160, and 320 γ/l was employed. The dose of 20γ was shown to be the minimum necessary in order to masculinize completely the gonads of the male genotypes at a stage (stage A in Fig. 9.5) when they are in the transitional female phase, 80 γ/l are necessary in order to masculinize also the genetic females: for this reason the lengths BC_3 and BD appear in the approximate ratio of 1 : 4 in Fig. 9.4. Only 5 γ/l are necessary to obtain complete masculinization of the male phenotypes while the masculinization of all the individuals (and thus also of the genetic females) is obtained with 320 γ/l at least. Thus the reaction curves for maleness and femaleness are far more distant than it was assumed in the original Goldschmidt's curves which receive nevertheless a striking confirmation from these experiments (Fig. 9.4).

As a comment to the above experiments on hormone action in gonadal differentiation it can be remarked that the paradoxal effect points to the close relationship existing between male and female steroid hormones which has

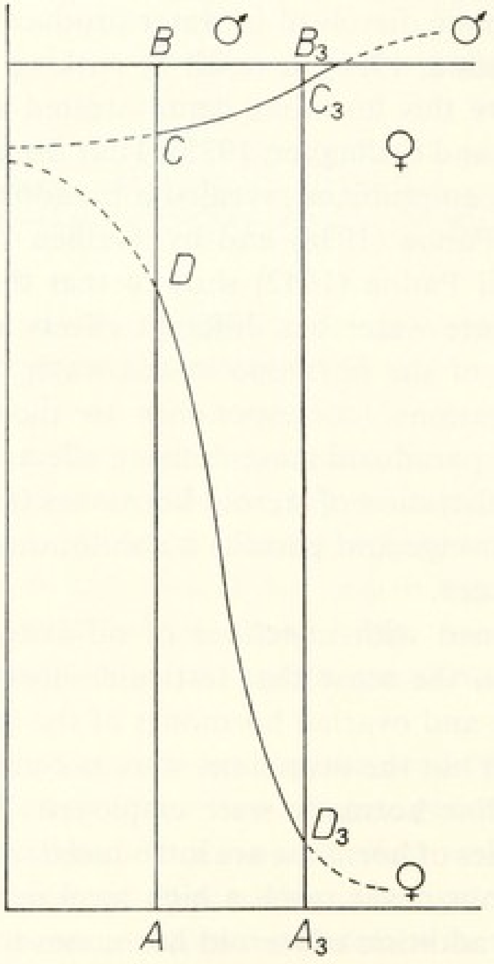


FIG. 9.4. The reconstruction of the male and female reaction curve in Padoa's experiments (Padoa, 1950).

found strong evidence both on the previously reported embryological research (Vannini, 1950) and on chemical investigation (Samuels, 1955). The reconstruction of Goldschmidt's curve, although it is not certain whether steroid sex hormones have a part in the normal process of embryonic gonadal sex differentiation, has finally given a quantitative value to some steps in the chain of reactions started by sex genes in the male and female genotypes.

The great quantity of male hormone that is necessary in order to obtain the masculinization of the older female genotypes accounts in Padoa's interpretation (1948) for the impossibility of obtaining sex reversal in adult amphibians by administration of high doses of hormones. They are nevertheless potent factors for the development of secondary sexual characters as showed by Galgano's experiments.

5. The Determination of the Secondary Sex Characters

Researches by Galgano upon the annual sexual cycle of the newt *Triturus cristatus carnifex* (1944, 1947) can give an idea of the complex mechanisms controlling some secondary sex characters (s.s.ch.) in amphibians (Fig. 9.5).

Among such characters eusexual and somatosexual characters must be distinguished. The former are developed under the influence of either male or female hormones produced by the gonads, the latter appear to be developed independently from the hormones secreted by the gonad. Ambosexual characters (Champy, 1920) are related to sexual activities but they appear to be common to both sexes.

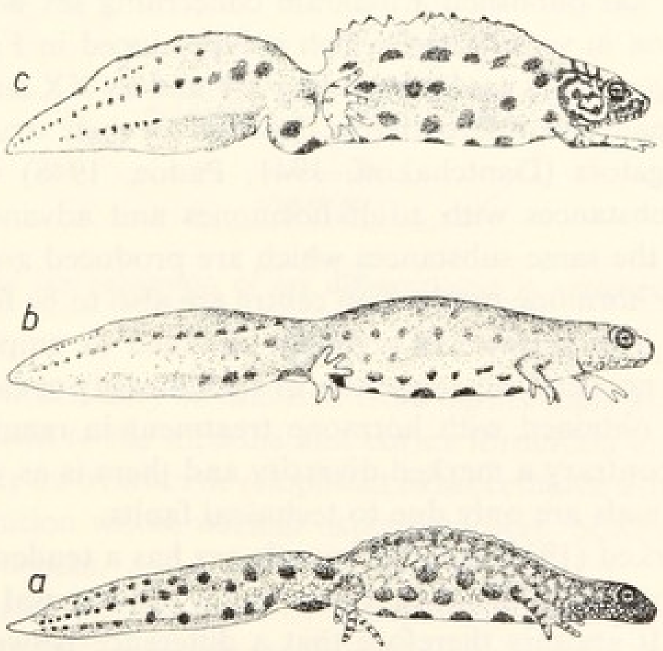


FIG. 9.5. Secondary sex characters in the annual sex cycle of *Triturus cristatus carnifex*: (a) a male individual in summer, (b) and (c) female and male individuals during the breeding season (Galgano, 1947).

The formation of a dorsal crest, the bright pigmentation and the strong secretory activity of the genital tract, which characterize the male newt during the breeding season are under the control of the testicular hormone. The turgidity of the cloaca and the secretory activity of the genital tract of female newts are on the contrary influenced by the ovarian hormone.

Part of the development of the crest in the male, the development of the tail and the peculiar aspect of the skin in both sexes are under the control of gonadotropic hypophysial hormones: male and female castrated individuals which were injected with hypophysial hormone during the season of sexual inactivity showed the same skin structure and the same form of the tail as whole individuals during the breeding season (Galgano, 1940).

Thus the determination of secondary sex characters appear to be rather complicated even in comparatively low vertebrate species. The experimental analysis shows the existence of eusexual as well as of ambosexual characters. Somatosexual characters will be revealed by the analysis of sex differentiation in other animals.

From the foregoing study it is nevertheless possible to give a general picture of the chain of mechanisms that lead from the primary action of sex genes to the sex differentiation of the adult phenotypes.

6. From the Sex Genes to the Sex Characters

It is now possible to make a rapid survey of the sequence of the sex differentiating processes in amphibia which will also give some ideas of how little is really known about sex differentiation among vertebrates.

Padoa (1948) has published a diagram concerning sex determination and sex differentiation in vertebrates, which is reproduced in Fig. 9.6. The male and the female genotypes are indicated by XY and by XX and the production of male and female sex differentiators depends on their structure.

Some investigators (Dantchakoff, 1941; Padoa, 1948) were inclined to identify such substances with adult hormones and advanced the working hypothesis that the same substances which are produced and diffused in the organism by the hormone production centre are also to be found in its molecular structure. The perfect sex reversals obtained in amphibians with the adult hormones are certainly suggestive of such identity or close similarity but the poor result obtained with hormone treatment in mammalian embryos suggest on the contrary a marked diversity and there is as yet no proof that failures in mammals are only due to technical faults.

Witschi remarked (1934) that the genetic sex has a tendency to show itself in adult parabiotic individuals that underwent sex reversal during the early parabiotic life. It appears therefore that a difference between the early sex inductors (medullarin and cortexin) and the sex hormones does exist. A histochemical investigation by Chieffi (1957) supports this view because methods that allowed localization of steroid substances in the inter-renal tissue failed to show their presence in the gonad at the stage of early sex differentiation. Although negative results cannot be considered decisive when completely dependable technical methods have not yet been developed it must be concluded that the view of a strong diversity between sex inductors and hormones appears at the present time the most probable assumption although the contrary hypothesis is more in accordance with the now prevailing views concerning primary gene actions.

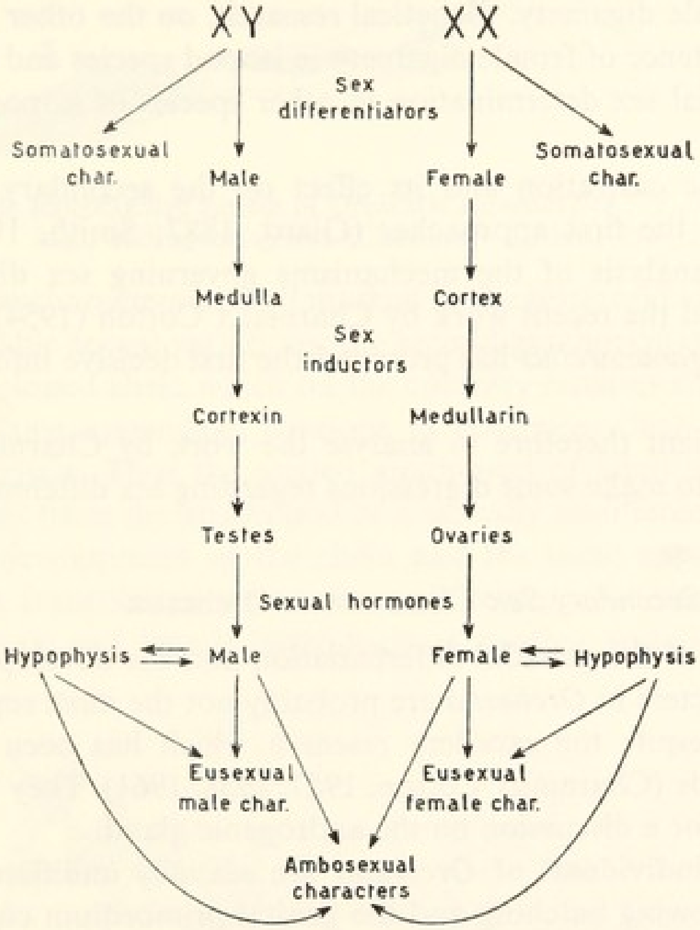


FIG. 9.6. Diagram of the succession of sex differentiators in vertebrates (Padoa, 1950).

Sex inductors, which may be different from earlier male and female differentiators, which lead to the medulla and cortex formation in vertebrates, induce a testicular or an ovarian development in accordance with the individual genotypic constitution whose normal expression may, however, be reversed by secondary influences.

Testes and ovaries produce male or female adult hormones which in turn influence eusexual male or female secondary sex characters either alone or by interaction with anterior hypophysial hormones. Ambisexual characters are developed by similar mechanisms.

Somatosexual characters, that have not been treated in the present section, as sex characters are mostly under the influence of the gonads among vertebrates, will be discussed in the section regarding sex determination in crustaceans.

Sexual Differentiation in some Crustacea

Recent reviews on the cytology of crustacea (Niiyama, 1959) enumerate the presence of XY, of XO, of multiple sex chromosome mechanisms, and of male and female digamety. Genetical research, on the other hand, has confirmed the existence of female digamety in isopod species and has demonstrated polyfactorial sex determination in other species of isopods and in copepods.

The parasitic castration and its effect on the secondary sex characters made possible the first approaches (Giard, 1887; Smith, 1913; Reverberi, 1944) to the analysis of the mechanisms governing sex differentiation in crustaceans and the recent work by Charniaux Cotton (1954) on the amphipod *Orchestia gammarellus* has provided the first decisive information on the subject.

It is convenient therefore to analyse the work by Charniaux Cotton on *Orchestia* and to make some digressions regarding sex differentiation in other crustaceans.

1. Gonads and Secondary Sex Characters in Orchestia

The models of the gonadal differentiation and the development of secondary sex characters in *Orchestia* are probably not the most representative for crustaceans, despite the excellent research which has been done on it at different periods (Charniaux Cotton, 1957, 1958, 1961). They form, however, the best basis for a discussion on the androgenic gland.

The young individuals of *Orchestia* are sexually undifferentiated in the first molts following hatching and the genital primordium consists of a thin cord of mesodermal cells enclosing several protogonia and extending from the second thoracic segment to the seventh thoracic segment. The germ cells of the differentiated male remain confined in the second, third and fourth segments and they extend back to the sixth thoracic segment in the differentiated female. The strand of cells in the last segment will form the sperm duct in the male and another strand of cells in the fifth thoracic segment will give rise to the oviduct of the female.

The structure of the adult gonads in *Orchestia* is thus basically the same in male and in female individuals although the ripe ovary is much more extended in length than the testis. Clusters of sperms and spermatids which are surrounded by a layer of big cells secreting a mucoid substance appear in the ripe male gonad. They are formed by a dorsal strand of numerous spermatogonia and spermatocytes at various stages of maturation. A similar strand with a smaller number of oogonia and oocytes rests upon a single layer of big oocytes which are surrounded by follicular cells during the last period of yolk formation. A sketchy representation of testis, sperm duct and androgenic gland is given here (Fig. 9.7) from a work by Charniaux Cotton.

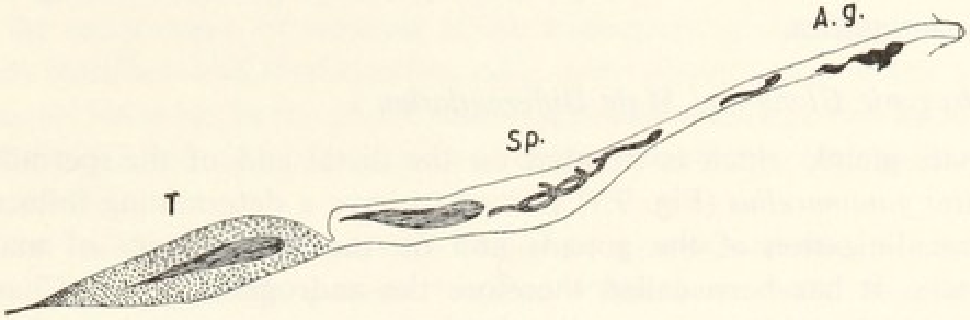


FIG. 9.7. The male reproductive system of *Orchestia gammarellus* : T., testis; Sp., sperms; A.g., androgenic gland (Charniaux Cotton, 1957).

Secondary sex characters are well marked in *Orchestia* and some appendages show considerable sexual variations: the second gnathopod of the adult male has a well developed chela which on the contrary remains small in the adult female. The same appendage develops in oostegite which bears marginal hairs in the female. Thus the second gnathopod of adult male individuals differ essentially from the gnathopod of a sexually undifferentiated specimen for the great development of the chela and the same appendage of adult females differs from the presence of a well developed oostegite. Figure 9.8 shows adult male and female individuals of another amphipod, *Gammarus*

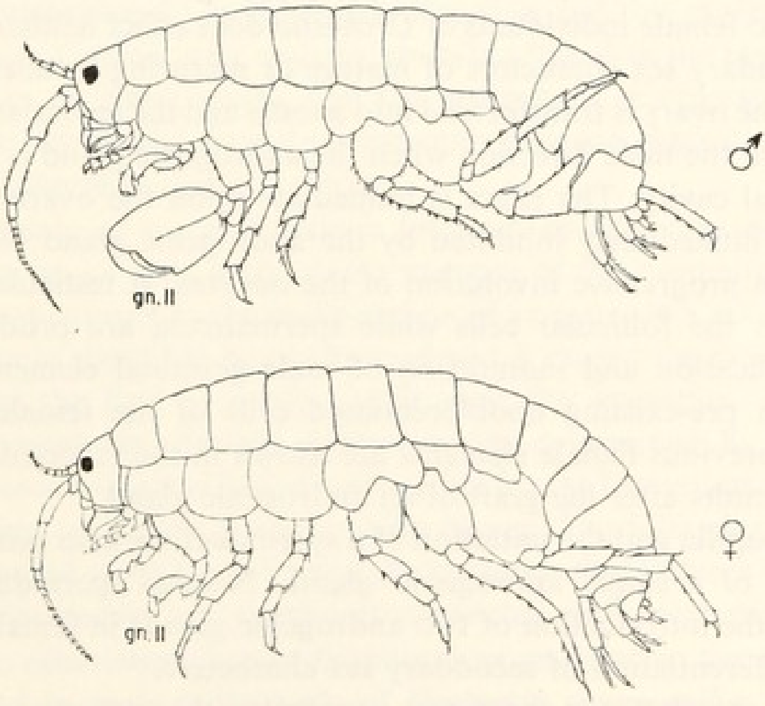


FIG. 9.8. Male and female individuals of *Gammarus pulex*: differences in the chelae of gnathopod II are evident.

pulex, where the differences in the chelae of the second gnathopod appear evident.

The full development of secondary sex characters is reached in *Orchestia*, as in all other crustaceans (Teissier, 1928, 1947; Drach, 1939) through stages of allometric growth that are separated by critical periods showing important discontinuities.

2. Androgenic Gland and Male Differentiation

A male gland, which is situated on the distal end of the spermduct in *Orchestia gammarellus* (Fig. 9.7) proved to have a determining influence in the masculinization of the gonads and on the development of male sex characters. It has been called therefore the androgenic gland (Charniaux Cotton, 1954) and precursor cells of this organ are present in early stages both in male and in female genotypes. Subsequent development takes place, however, only in males: the cells, which were linearly arranged in the newly formed parts of the gland, increase in size and become multinucleated and vacuolated until the nuclei become pycnotic and the cells degenerate. The androgenic gland is therefore of the holocrine type.

The work of numerous authors (Legrand, 1958; Katakura, 1959, 1961; Legrand and Juchault, 1960) showed that the androgenic gland is present also in other amphipods, in isopods, in stomatopods and in decapods where it may assume a somewhat different structure and also quite different positions. In oniscoids metameric androgenic glands are found at the cephalic end of the testes.

Charniaux Cotton demonstrated in her experiments that the implantation of a testis into female individuals of *Orchestia* does effect neither the primary nor the secondary sex characters of mature or maturing females, but on the other hand, the ovary is transformed into a testis and the secondary sex characters develop in the male direction when the androgenic gland is implanted in the pericardial cavity. The effect is immediate upon the ovaries where yolk formation is immediately inhibited by the androgenic gland implant and is followed by a progressive involution of the oocytes. A testicular epithelium soon replaces the follicular cells while spermatozoa are produced after a rapid multiplication and maturation of male germinal elements that have evolved from pre-existing undifferentiated cells of the female gonad. No trace of the previous female elements are shown in the reversed gonad three or four intermolts after the graft of an androgenic gland.

A genital papilla and the initiation of a spermduct are also produced by the implantation of a single androgenic gland. Normal spermducts are only formed with the introduction of two androgenic glands in female individuals beginning differentiation of secondary sex characters.

The male secondary sex characters concerning the structure of the gnathopods and of the seventh pereiopod are gradually acquired in the course of the post-operative intermolt periods just in the same way as they are acquired in the normal males. Testes degenerate and ovaries can be implanted without modification in male individuals that have been deprived of the androgenic gland. If the gnathopod is removed in such males they regenerate in undifferentiated form.

Thus the experiments of removal of the androgenic gland fully complete the results obtained with implantation, and, as the absence or presence of the testes has no influence in the above results, the androgenic glands appear to secrete the only hormone producers which are responsible for male differentiation in *Orchestia*.

Reversed sex phenotypes have been employed in crosses tending to establish the type of sex digamety: genetic females which have been transformed into males, cannot operate normal fertilization because their spermducts are usually not open but their spermatozoa have been used to artificially fertilize freshly laid eggs. Only ten female offspring have been obtained so far by this method (Charniaux Cotton, 1960). Such early results are strongly indicative for a homogamety of the male sex although more experiments are necessary in order to conclude the existence of female digamety in *Orchestia*.

3. The Spontaneous Female Differentiation of the Gonads and the Hormonal Action of Ovaries

The removal of the androgenic glands in very young males is followed by oogenesis in the testis of *Orchestia*. As this female differentiation takes place in the absence of androgenic hormone, primary germ cells were supposed to differentiate spontaneously in the female direction and this explains, according to Charniaux Cotton, why oocytes are often found in the testes of decapods. It appears, in other words, that the protogonia of crustaceans evolve in female directions if they do not receive male homone regardless of genetic constitution (Charniaux Cotton, 1961).

The ovariactomy of a ripe female is followed by the replacement of the ovigerous hairs by juvenile hairs on the margins of the oostegites. The converse experiment consists in the implantation of an ovary in a male individual whose androgenic gland has been removed and it caused the development of oostegites after the first or second molt after the operation. It has been deduced that secondary female characters are determined by an ovarian hormone. An ovarian factor is also responsible for the formation of ovigerous hairs at the time of a molt preceding egg laying. The follicular cells of the ovary are supposed to be the source of the hormone that induces the development of such temporary sex characters connected with incubation.

The decisive experiment in this direction was made when testes of *Talitrus* were implanted in male individuals of *Orchestia gammarellus*, which were deprived of their androgenic glands. The implanted testes changed into ovaries and secondary female sex characters appeared in the genetic males.

The demonstration of the secretion of a female hormone from the ovary has led to the idea that sex genes of Malacostraca act by determining the presence or absence of the androgenic glands (Charniaux Cotton, 1963). The suppression of the androgenic gland by female factors resembles the situation in the sex determining mechanism of *Melandrium*.

An eyestalk hormone takes part in the control of the reproductive cycle of the females, as first shown by Panouse in his experiments on the shrimp *Palaemon serratus* (1943, 1946) and its effect consists probably in blocking yolk formation of oocytes (Stephens, 1952).

It appears therefore that protogonia tend to differentiate in the male direction and that in normal development their tendency is not checked in the female genotypes but it is reversed by the androgenic gland in the male genotypes. Young and ripe ovaries seem to function as endocrine glands so that secondary sex characters are determined by the androgenic gland in the male, by the ovaries in the female phenotypes. The role of the X organ sinus gland complex, which is located in the eyestalks, seems to be confined to a control in reproductive but not in differentiating processes.

4. *The Determination of Male Intersexes*

Experiments with the androgenic gland have also helped in explaining the presence of intersexual males which are found in certain populations of *Orchestia* (Charniaux Cotton, 1957c). They possess normal testes but develop oostegites and gnathopods with juvenile characters. If the androgenic gland is removed from such intersexual males and is implanted into a normal male, abnormal gnathopods and oostegites are developed by the host. This result explains also, in Charniaux Cotton's interpretation, why the testes are always normal (Charniaux Cotton, 1953) in male specimens that show secondary sex characters of intermediate form.

It has been shown that the above type of intersexuality is hereditary although it has not been investigated whether it is due to a sort of genic balance or to some other possible genetic mechanisms.

Comparisons with the mechanisms of vertebrate sex differentiation show some similarity consisting in the induction of the male sex in the germ cells by means of somatic organ. Humphrey's experiments actually demonstrated somatic induction of sexuality in amphibians both for the male and for the female sex. Somatic sexualization of the germ cells takes place also in male amphipods and probably in all male Malacostraca.

Current research shows on the other hand that differentiation of primary as well as secondary sexual characters is determined in Malacostraca by the same mechanisms of *Orchestia* and some results that formerly appeared rather puzzling are now brilliantly explained on the basis of the work in *Orchestia*.

5. *Androgenic Gland, Parasitic Castration, Intersexuality and Hermaphroditism in* Malacostraca

Researches on decapods that are parasitized either by Rhizocephala or by isopods showed that the secondary sex characters of the males are reversed to the female type and that the gonads are generally much reduced and sometimes contain a few oocytes. The study of the parasitic castration was initiated by Giard (1887) on crabs parasitized by *Sacculina* and soon a large series of observations on this subject led to the development of theories regarding sex differentiation. Smith (1913) supposed that sexual differentiation is related to fat metabolism, an idea that was later applied by Orton (1927b) to the alternation of sexual phases in oysters. Giard himself believed that the atrophy of the gonads is the cause of the feminization of the secondary sex characters in parasitized males. The theory that secondary sex characters in males are not determined by the testes was advanced by Courrier (1921) and it was eventually demonstrated as a consequence of Charniaux Cotton's experiments which showed that in some of the crabs parasitized by *Sacculina* a hypertrophy of the androgenic gland takes place (1954). Veillet and Graf discovered later (1958) that in three species of crabs and in a pagurus parasitized by Rhizocephala a degeneration of the androgenic gland takes place and it is accompanied by a feminization of the secondary sex characters. The problem of parasitic castration appears thus very near to a complete solution in consideration of the action of the parasite upon the androgenic gland. The discovery of this mechanism does not rule out, however, the validity of former explanations that pointed to the fact that the balance between sex genes is more or less stable depending on the species (Reverberi, 1944; Veillet, 1945) and therefore their sex characters can be more or less modified by the action of the parasites.

The control of the development of the androgenic gland in different sex genotypes appears to be a promising field for future investigation in the sex physiology of crustaceans and the study of intersexual phenotypes that are frequently observed among the females of the isopods *Porcellio dilatatus*, *Porcellio maculatus* and *Porcellio pruinosus* have provided excellent material for the study of correlation between sex genotypes and sex characters (Legrand, 1956; Shimoizumi, 1956, 1958) under given environmental conditions.

The discovery of an important endocrine mechanism does not exclude in crustaceans, the existence of primary sex differentiators that exert their influence, on the basis of their quantitative balance, by developing the androgenic gland in the male genotypes and by inhibiting its development in the female genotypes.

An intersexual condition is also shown by male amphipods of the species *Talitrus saltator* and *Orchestia mediterranea* whose anterior parts of the gonad contain unripe oocytes which begin differentiation before spermatogenesis. The situation is therefore very similar to that of the male genotypes of the undifferentiated races of *Rana* or to that of male *Bufo*. It seems to indicate a labile condition of the sex genotypes.

The succession of the sexual phases in the protandrous hermaphrodite shrimp *Lysmata seticaudata* is also correlated with the regression of the androgenic gland as the ovarian part of the gonad reaches maturation soon after the inhibitory activity of the gland has finished (Charniaux Cotton, 1958). The implant of androgenic glands in female phase individuals induced masculinization of the external sex characters and partial reversal to the male sex of most gonads. The androgenous hormone exerted its action upon the protogonia according to a postero-anterior gradient in such a way that the anterior part of the gonad has retained its ovarian aspect in a few treated individuals (Charniaux Cotton, 1961). The physiology of sex reversal has thus been successfully approached in higher crustaceans.

The problems regarding sex differentiation of crustaceans where, as in *Ione*, the reaction norm of the sex genotype allows the free play of the external factors or in species where an integration of external with genetical sex conditions plays a predominant role, have not been approached yet.

The problem of complementary males is also still very obscure and only working hypotheses have been advanced concerning sex in heterogonic cycles of crustaceans.

The discovery of the androgenic gland represents, however, an important step in understanding the physiology of sex differentiation both in crustaceans and in other metazoa.

CHAPTER 10

MALE HAPLOIDY AND ARRHENOTOKOUS PARTHENOGENESIS

HAPLOID bees and wasps are male and diploid ones are female but it is not fully clear yet how sex is determined in the course of this peculiar haplodiploid cycle. The honey bee provides the most widely known example of male haploidy which is associated with male producing parthenogenesis.

Apis mellifica, Arrhenotokous Parthenogenesis and Male Haploidy

The common bee *Apis mellifica* is differentiated into three kind of individuals, the males or drones, the queens or functional females and the workers which are sterile females. The functional females normally live 4 and 5 and even 6 yr, the workers generally live 5 or 6 weeks and the males only appear in spring. Copulation takes place only once in the life of a female bee during what is known as the marriage flight. The young larvae are fed uniformly with a secretion of the lateral pharyngeal glands during the first 3 days of their life but the larvae destined to produce males and workers are fed upon honey and partially digested pollen from the fourth day of life on. The larvae of the queens, on the other hand, continue to be nourished upon the same diet as the first 3 days throughout life.

Differences in ovarian development as well as the other characters that distinguish queens from workers are thus determined by differences in diet. Sex, on the other hand, is determined neither through environmental influences nor through a genetic mechanism resembling those discussed before. The first information concerning sex determination in bees was given more than a century ago by Dzierzon (1848) on the basis of his observations on bees which produced male progeny. Dzierzon showed that eggs laid by virgin queens give rise only to male individuals. No females are produced by workers that have become able to develop functional ovaries in the absence of the queen but have not been fertilized. Old queens, whose provision of spermatozoids is over are no longer able to produce female progeny and only produce male offspring.

Dzierzon deduced from his observations, which were confirmed by later experiments, that males develop from eggs that develop parthenogenetically and females are formed from fertilized eggs.

Bees thus provided the first known example of arrhenogenous parthenogenesis, where unfertilized eggs produce male individuals only. As both fertilized and unfertilized eggs of bees are capable of development they are said to show facultative parthenogenesis.

Blockmann (1889) demonstrated that the egg of *Apis mellifica* emits two polar bodies and Nachtsheim (1913) showed later that normal meiosis takes place leading to the formation of four haploid nuclei with sixteen chromosomes each. Female bees have therefore thirty-two chromosomes and recent claims (Manning, 1948, 1950) that female have thirty autosomes plus one X chromosome have been disproved (Ris and Kerr, 1952; Sanderson and Hall, 1951). Observations in a number of other species have shown that oogenesis is absolutely normal in cases of arrhenogenous parthenogenesis which gives rise to haploid males.

Spermatogenesis of the haploid males of *Apis* has been accurately described in a famous work by Meves (1907). During the stage corresponding to

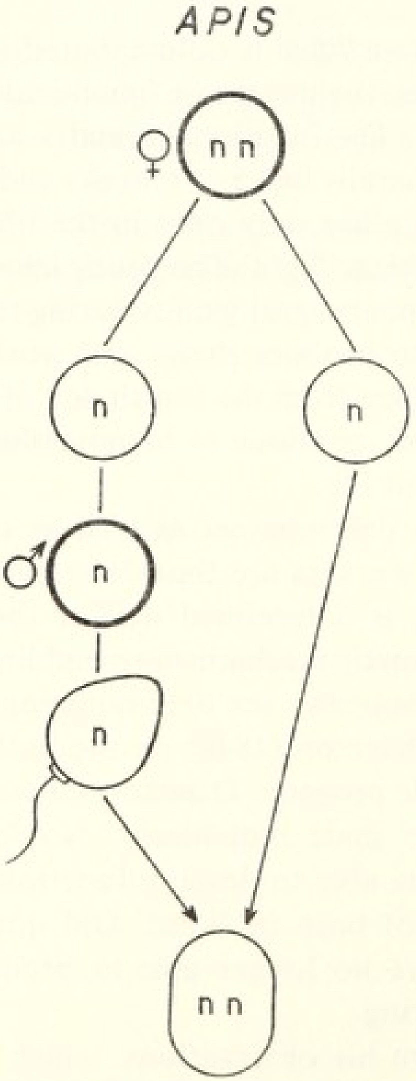


FIG. 10.1. The arrhenotokous parthenogenetic cycle of *Apis*. The thick walled circles indicate male or female individuals.

the first maturation division a figure resembling an achromatic spindle appears (Fig. 10.2) but no nuclear division takes place and the nuclear membrane is not dissolved. A cytoplasmic bud, which can be compared to the cytoplasmic portion of the secondary spermatocyte, detaches itself from the spermatocyte and degenerates. The second maturation division resembles a normal mitosis but the two spermatids resulting from this division are very different in size and the smaller of the two degenerates. As a result of such processes a single functional sperm is originated from one primary spermatocyte and it carries the same haploid number of sixteen chromosomes.

The haplo-diploid cycle of *Apis*, which can be taken as a model for similar cycles of arrhenotokous Hymenoptera is represented in Fig. 10.1 where diploid female (nn) and haploid male (n) individuals are represented by thick circles, haploid eggs by thin circles, and the fertilized egg (nn), giving rise to female individuals, by an oval-shaped figure. The sperm is represented, in the present and in similar figures, by the conventional outline of a nemasperm.

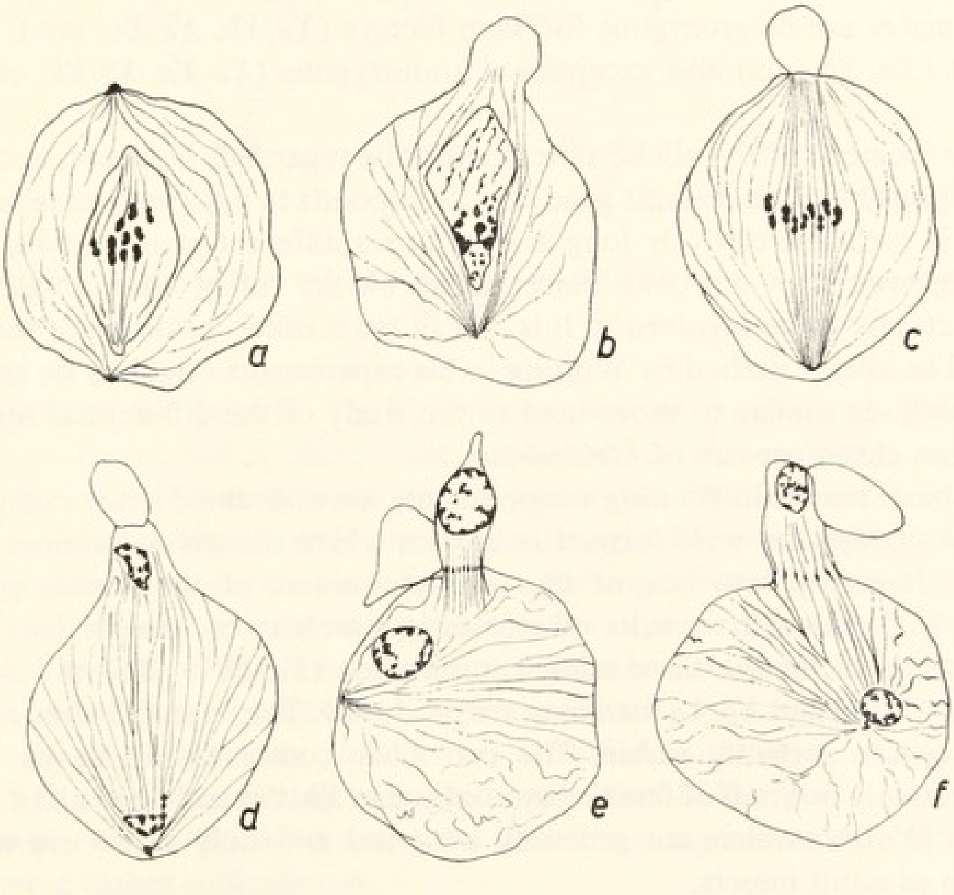


FIG. 10.2. Maturation divisions of the spermatocytes of *Apis* (Meves, 1907).

A similar type of spermatogenesis takes place in other aphids like *Bombus* (Meves, 1904) and *Xylocopa* (Granata, 1908) with the formation of a single spermatid from each spermatocyte. Two spermatids of equal size, and consequently two sperms, are formed from one spermatocyte in wasps

(Meves, 1904), in ants (Lams, 1908), in *Habrobracon* (Whiting, 1918; Torvik Greb, 1935) and in a number of other hymenopters.

The male haploidy of Hymenoptera, where arrhenotoky is most widespread, implies a peculiar genetic mechanism of sex determination which has been investigated and only partially explained through the work of Whiting and his collaborators on *Habrobracon juglandis*, a braconid parasite on the larval stages of some moths.

The So-called Complementary Sex Determination in *Habrobracon*

Research on gynandromorphs and on the inheritance of a sex-linked gene lead Whiting (1928 a,b) to the conclusion that the diploid females carry the X and Y sex chromosomes and that the haploid males carry either the X or the Y chromosome. Exceptional XX and YY individuals are males and sterile.

A series of nine multiple alleles, which are designated *Xa*, *Xb*, *Xc*, etc. to *Xi*, was discovered by Whiting (1940, 1943). They are regarded as sex determiners and females are heterozygous for such factors (*Xa*/*Xb*, *Xa*/*Xc*, etc.). Hemizygotes (*Xa*, *Xb*, etc.) and exceptional homozygotes (*Xa Xa*, *Xb Xb*, etc.) are males.

Each member of the allelic series cannot be regarded, however, according to Whiting (1945), as a single gene but corresponds to a chromosome segment which is perhaps relatively long and contains different genes, among which crossing-over does not take place. Gene blocks rather than single genes seem actually to be involved in this and in most other instances of sex digamety. The alleles studied by Whiting in his experiments ought to be analysed with methods similar to those used in the study of the differential segments in the sex chromosomes of *Melandrium*.

The basic results in Whiting's experiments were obtained when exceptional viable homozygotes were formed in crosses where the sex determiner of the male is identical with one of the sex determiners of the female partner. Figure 10.3 shows the results of crosses in which three or only two alleles are introduced. In the three allele combination (*Xa*/*Xb* × *Xc*) only females with the *Xa*/*Xb* and *Xb*/*Xc* formulas are produced. They are all heterozygotes, and they are perfectly viable. The two allele combination (*Xa*/*Xb* × *Xa*) produces only one half of female heterozygotes (*Xb*/*Xa*) and one half of homozygotes (*Xa*/*Xa*) which are generally not vital and only a few are able to develop as adult insects.

Such exceptional individuals differentiate as males and their diploid constitution has been demonstrated by Whiting through crosses between females that show a recessive allele for a given character and haploid males that carry the wild type allele for the same character. The haploid male sons will show therefore the recessive character and the diploid ones will show on the contrary the wild type character. The high lethality of homozygotic male individuals in the two allele combinations reduces, however, to nearly one half the number of the F_1 progeny.

Whiting concluded from his experiments that sex determination in *Habrobracon* has a complementary character with heterozygotes showing more of the dominant traits determined by female genes and males showing more of the recessive characters determined by male genes. Such genes are located, according to Whiting, in the segment where crossing-over does not take place and therefore various genes influencing secondary as well as primary sex characters act as a single series of allelic factors although sex determination can be regarded as polygenic.

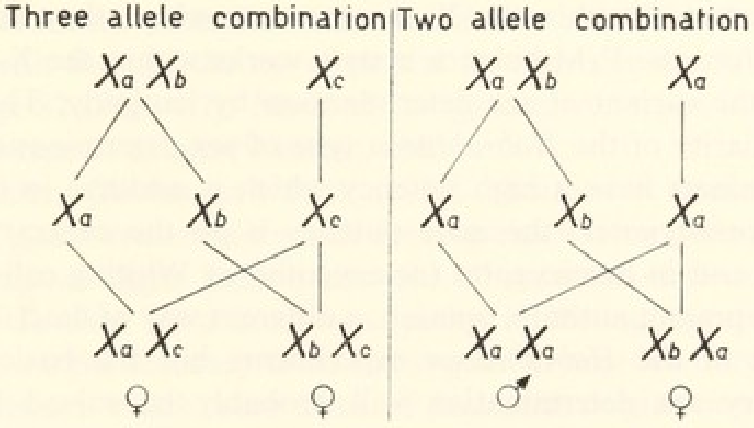


FIG. 10.3. Comparison of the two and three allele combinations in *Habrobracon* (after Whiting).

A dominant sex gene F_a, F_b, F_c and a number of recessive sex genes m_a m_b, m_c, or their normal alleles, are present in the chromosomal segments X_a, X_b, X_c . . . . Heterozygotes have therefore two F which are epistatic to the homo or heterozygous *m*. Homozygotes have an FF which is hypostatic to a number of homozygous *m* because it is not acting differently from the single F which is hypostatic to the many hemizygous *m* in the haploid individuals.

The low vitality of the homozygous individuals seems also to indicate, in the present author's opinion, the existence of some kind of incompatibility system whose factors may perhaps be identified with some of the genes localized in the "sexual" segment. The separation of the genes responsible for the low vitality of the homozygotes from the sex genes, if possible at all, will prove a rather difficult task.

The major problem posed by the work on *Habrobracon* consists, however, in trying to explain in terms of balance of genic action the so-called complementary sex determination, that is why the chromosomal segments of Whiting are male determining when in the hemizygous or in homozygous condition and are female determining when in heterozygous condition. The problem is actually not restricted to the very specialized type of sex determination of

Hymenoptera but concerns also sex determination in *Tisbe* and probably in a number of other Copepoda (see Chapter 6). The only difference seems to be that sex genes are restricted in *Habrobracon* to different segments of sex chromosomes and are located in autosomes in *Tisbe*.

The balance theory of sex, both in its former implication as a balance between sex genes located in the sex chromosomes and autosomal sex genes and in its recent extension concerning the balance between female and male autosomal genes seems to have a general application and Goldschmidt tried (1955) to interpret Whiting's results within the limits of a comprehensive theory. According to Goldschmidt the type of determination in *Habrobracon* is an extreme variant of the general scheme with the male and female sex determiners concentrated within the X chromosome where they are completely linked. Therefore the F/M balance system works within the X chromosome and permits the variant of sex determination by haploidy. The second important peculiarity of the *Habrobracon* type of sex determination is that the female determiners have a high potency which is additive in heterozygotes but not in homozygotes; the male potency is on the contrary additive in homozygotes and in hemizygotes (or azygotes, as Whiting calls them). This is only, in the present author's opinion, a different way of describing the facts demonstrated in the *Habrobracon* experiments but the basic problem of complementary sex determination will probably be solved by a further analysis of the gene blocks in *Habrobracon* and by a detailed investigation of the polygenic system in *Tisbe*.

The condition of the so-called complementary sex determination of *Habrobracon* can be located at the extreme end of series of systems of sex determination which begins with sex polygamety, where female and male determiners are distributed in the whole chromosome complement and culminates in the concentration of all sex determiners within the sex chromosomes.

A slightly different theory was advanced for bees by Da Cunha and Kerr (Kerr and Laidlaw, 1956) who suggested that genes for femaleness have a cumulative effect which is lacking in the male genes. As a consequence the balance between the genes would be determined in the following way: haploidy—MF—male sex, diploidy—2FM—female sex. This mechanism does not explain the appearance of male homozygosis whose existence, however, has been demonstrated only on limited parts of the body of the honey bee.

Sex Determination in Honey Bees

Matings of closely related individuals showed in the bees the existence of a series of haploviable alleles that proved to be lethal in homozygous condition (Mackensen, 1951) and mother–son matings resulted also in low viability.

Mackensen advanced the hypothesis of a close connexion of the lethal alleles with sex determination and based his view upon the consideration that it is difficult to imagine the survival of the series of lethals demonstrated in *Apis mellifica* unless it is associated with a process of such fundamental importance as sex. The honey bee has developed peculiar behaviour patterns to favour outbreeding as inbreeding often brings about low viability. Some kind of incompatibility factors appears to be concerned in the reproductive system of bees and no homozygous males could be obtained.

The multiple sex allele hypothesis could, however, be demonstrated in the absence of the diploid males and evidence in this direction was obtained through the work on gynandromorphs which may arise in various ways.

Mackensen found one individual in which female parts arose through fertilization and male parts had maternal characters. This observation is in accordance with the old theory of partial fertilization which has been discussed at page 82 (Fig. 4.7). Most of the gynandromorphs in *Habrobracon* had the same type of origin (Whiting, 1927).

Rothembuhler, Gowen and Park (1952) obtained a strain with a high percentage of gynandromorphs which develop either from unfertilized or from normally fertilized eggs. Male parts are of paternal chromosomal origin because they develop from accessory sperms in the egg cytoplasm. Female parts develop on the contrary from the zygote. Gynandromorphs with male and female tissues which were both of maternal origin were obtained by Tucker (1958) in the course of experiments with unmated queens that were heterozygous at one or more loci. Those females produced a few gynandromorphs and even numerous females from over-ripe eggs that develop without fertilization. In Tucker's interpretation the spindle of the first meiotic division lays parallel to the egg surface instead of projecting into the egg. As a result of the second maturation division two female pronuclei and two polar bodies instead of a single pronucleus and three polar bodies are produced. Caryogamy takes place between the two pronuclei and gives rise to the female tissue while male tissues of the gynanders are developed from the remaining haploid nuclei. Purely female individuals are generally derived from this unusual type of regulation of the chromosome number in the parthenogenetic eggs of the honey bee. It must be remarked that similar processes occur normally during the maturation of the parthenogenetic eggs of the moth *Solenobia lichenella* (Seiler, 1923) and of aphids (Cognetti, 1961a,b).

Male diploid tissue obtained through fertilization has eventually been found by Rothembuhler (1957) in mosaic male tissue of gynandromorphs. This seems to be the only viable combination for the diploid male genotype and it explains why the search for homozygous males has been unsuccessful in *Apis*.

Drescher and Rothembuhler (1964), who employed a gynandromorph producing line and genetic markers, produced by close inbreeding 66 mosaic drones which showed eye tissue of zygotic origin but no honey bee male of complete zygotic origin, as those obtained in *Habrobracon*.

Mackensen's data on allelic lethals and Rothembuhler's discovery of diploid male tissue represent therefore the only evidence in favour of a type of sex determination which is quite similar to the multiple allele mechanism discovered in *Habrobracon*.

Researches on *Habrobracon* and especially on bees show that complementary sex determination is represented in species where outbreeding is prevalent.

A system where incompatibility effects are absent, must take place on the other hand among parasitic hymenopterans which have a close inbreeding. The Proctotrupid *Telenomus fariae* is an egg parasite in which brother–sister copulation takes place before leaving the egg shell of the host (Dreyfus and Breuer, 1944). The work of Schmieder and Whiting (1947) on the wasp *Melittobia* showed that virgin females produce eggs that develop into males which eventually may mate their own mother and produce quite normal diploid progeny. Schmieder and Whiting consider the multiple sex allelism as the primitive pattern of sex determination in Hymenoptera, from which a different pattern has developed in species showing close inbreeding.

It is evident therefore that parasitic arrhenotokous Hymenoptera have different patterns of sex determination that must be closely influenced by their reproductive biology.

Heteropycnosis and Male Haploidy in Coccids

1. The Gonochoric Species

Male haploidy occurs among Iceryini (Hughes Schrader, 1948) and among Diaspididae (Brown and Bennett, 1957) two groups of scale insects or coccids. Coccids have only been investigated cytologically but comparative research on members of different tribes and sub-families has yielded extremely interesting data concerning the origin of male haploidy.

The chromosomes of coccids, like those of most species of Homoptera and Heteroptera, have diffuse centromeres which can be also regarded as polycentric centromeres.

Both sexes are diploid in the tribe Llaveini where an XO sex chromosome mechanism has been demonstrated (Hughes Schrader, 1942).

The lecanoids show a peculiar chromosome cycle which differs both from the typical diploidy of Llaveini and from haplodiploidy of Iceryini and Diaspididae. No sex chromosome mechanism could be cytologically demonstrated.

Hughes Schrader (1935) found twelve chromosomes in the diploid set of *Pseudococcus acericola*, which provides a typical example of the chromosome cycle of Lecanoinae. No sex chromosomes have been detected but sexes can be recognized cytologically as early as blastula stage because one haploid set of chromosomes shows extreme condensation in the somatic nuclei of the male and such a difference is absent in the female. The chromosomes of the heteropycnotic set are clumped at one side of the nucleus and the others are uniformly distributed in the rest of the nucleus and it is impossible to establish whether the heteropycnotic set is paternal or maternal in origin. Ovogenesis is normal but no bivalents are formed during spermatogenesis and no crossing-over can take place in the male. The two chromosome sets cannot be distinguished at first metaphase during spermatogenesis but one of them becomes heteropycnotic again during interkinesis. Only the heteropycnotic set is connected with the spindle during the second maturation division and the chromosomes of the other group remain in a diffuse condition. Two nuclei are formed by the diffuse elements and develop into sperms while the chromosomes of the heteropycnotic set soon degenerate.

Thus the haploid set of chromosomes, that has become heterochromatic during the blastula stage of the males, is eliminated during spermatogenesis. Schrader and Hughes Schrader (1931) remarked that the males of Lecanoinae breed as haploids although they have a diploid chromosome number. Virtual haploidy may lead to actual male haploidy through a degeneration of the heteropycnotic set ending with its complete elimination.

A transition to true male haploidy has actually been discovered (Brown and Bennett, 1957) in the Diaspine scale *Pseudocalaspis pentagona* whose males are haploid ($n = 8$) and females diploid. Both males and females arise, however, from fertilized eggs and males become haploid because an haploid set remains undivided during the later egg divisions until it is eliminated at a stage corresponding to the differentiation of the heterochromatic set in the Lecanoinae. X-ray treatments have conclusively demonstrated that the eliminated set is of paternal origin.

The final step, depending on the ability of eggs to develop without fertilization, has led to true male haploidy and arrhenotoky among the Iceryini, which were early investigated by the Schraders (1926). Females develop from fertilized eggs and have two pairs of chromosomes in the five species investigated so far, males are haploid and, contrary to the males of Lecanoinae and of Diaspinae, develop from unfertilized eggs. Spermatogenesis takes place with a single equational maturation division like in the males of Diaspinae scales.

Although cytological research has provided brilliant results concerning the evolution of haploidy, very little is known concerning the actual method of sex determination in scale insects with the Lecanoine, Diaspine and Iceryine types of chromosomes cycle. In the lecanoid *Pseudococcus citri* James (1937) found evidence that environmental factors can strongly influence the sex ratios which vary greatly with ageing of the mothers prior to mating and alter the sex ratios in favour of the male progeny. A similar alteration due to the ageing of the mother or the X-ray treatment was also reported by Brown and

Bennett (1957) in the already mentioned work on the Diaspine scale insect *Pseudolacaspis pentagona*. The work on *Pseudococcus* (or *Planococcus) citri* has been recently resumed by Nelson Rees (1960, 1962), who confirmed the increase of the sex ratios in favour of males with ageing of the females. Raising of temperatures produced an increase in the proportion of males and a decrease in the number of offspring. Very interesting results were obtained with X-ray treatment which demonstrated that ageing of the female prior to mating changed the nature of the sensitivity of the ovarioles thus altering the sex ratio of the surviving offspring.

High dosage paternal irradiation affected the fertility of the sons and suggested the existence of fertility factors in the heterochromatic set.

A sex predetermination appears to be demonstrated in *Pseudococcus citri* and the effect of the treatment on the sex ratio, depends, in Nelson Rees' interpretation, from the stage of meiosis of the oocyte and from the nature of the egg cytoplasm at fertilization. This resembles the pioneer observations of Seiler on *Talaeporia* although sex chromosomes have not been detected in the Lecanoid scale insects. Environmental influences and the conditions of the cytoplasm certainly exert a strong influence in the sex determination of the coccids but their precise knowledge can only be considered as preliminary to the genetic experiments that, in the opinion of the author of this review, are still needed in order to discover the mechanism which is ultimately responsible for sex determination in coccids and which may be similar to the mechanism which has been discovered in arrhenotokous Hymenoptera.

A secondary specialization of male haploidy in coccids has resulted in the hermaphroditism of three species; the cycle has been best investigated in one of them (*Icerya*).

2. *Male Haploidy and Secondary Hermaphroditism of* Icerya purchasi

A few haploid males are found in *Icerya purchasi*, a well-known pest of orange trees. Hermaphrodites externally resemble females of related species but they possess a hermaphrodite gonad, whose peripheral parts are mostly ovarian and the central part is testicular (Pierantoni, 1913). The somatic tissues and the ovarian part of the ovotestis are diploid with two pairs of chromosomes, the testicular part of the gonad is haploid with only two chromosomes in the spermatogonia.

As Hughes Schrader demonstrated in her investigations (1925, 1927), oogenesis is quite normal and spermatogenesis takes place with a single maturation division as in other Iceryinae. Mature eggs and sperms are both haploid and fertilized eggs produce hermaphrodite, unfertilized eggs produce male individuals.

Haploid germ cells begin to appear in the diploid hermaphrodite individuals soon after hatching and they form the centre of the gonad from which the testicular parts develop. Hermaphrodite individuals can thus be considered chromosomal mosaics in which the male group of cells in haploid condition is similar to the haploid gametophyte wrapped within the diploid tissues of the sporophyte of angiosperms. It is unfortunate that the details of the reductional process from which the haploid male cells have arisen are not known yet.

Hermaphroditism of *Icerya purchasi* has evolved from a primitive condition of the XO : XX sex determining mechanism. The disappearance of one set of autosomes in the males has led to the establishment of haploidy which is present in all the Iceryini that have been cytologically investigated. The last step has consisted in the transformation of the female of *Icerya purchasi* (and of other species of the genus) in a chromosomal mosaic where gynandromorphism has not presented the development of mature sperm and oocytes in the same individual.

The peculiar type of hermaphroditism in *Icerya purchasi* strongly resembles the case of complementary males in Cirripedia which, however, form a large group where hermaphroditism is constantly observed and does not represent a secondary acquisition.

Male Haploidy and Arrhenotokous Parthenogenesis in Animals Other than Hymenoptera and Homoptera

Male haploidy and arrhenotokous parthenogenesis have been observed in two other orders of insects other than Hymenoptera and Homoptera, that is in Thysanoptera and in Coleoptera. Similar cycles have been demonstrated also in *Acarina* (Arachnida). Haplodiploidy in the life cycle of rotifers needs re-investigation.

Male haploidy combined with paedogenetic reproduction has been demonstrated in *Micromalthus debilis*, the only representative of the coleopteran family Micromalthidae. Scott (1936–1941) demonstrated that this beetle has four kinds of reproductive females: (1) an adult female, (2) a paedogenetic larva producing female progeny, (3) a paedogenetic larva producing male progeny and (4) a paedogenetic larva producing both male and female progeny. Males are haploid ($n = 10$) and the adult females are diploid ($2n = 20$) and no sex chromosomes have been detected.

A unipolar spindle is generally formed during spermatogenesis. Neither chromosomes nor cytoplasm divide as a consequence of the first spermatocyte division which is consequently wholly abortive. The second division resembles a normal mitosis and two apparently normal sperms are ultimately originated from each spermatocyte.

Adult male and female *Micromalthus* have been observed in North American strains but no males have been detected in South African strains and it has not been ascertained whether fertilization actually takes place between males and adult females even in the race where they are produced. It has been supposed therefore that the adults are sterile and functionless and that only larvae carry on reproductive processes.

Scott showed that the arrhenotokous larvae usually produce a single adult male because its larva devours the mother. When this is prevented new eggs develop in the ovary of the mother and they give rise to females. This has been interpreted as a proof that sex of *Micromalthus* is determined by intrinsic or extrinsic environmental conditions and not by the maternal genotype. Only genetical investigation, which is entirely lacking at present, will establish whether the remarkable sex variability of the paedogenetic larvae of *Micromalthus* corresponds to the existence of multiple sex genotypes.

Arrhenotoky is found in the Acarina belonging to the families Tetranychidae, Tarsonemidae, Laealaptidae and Macrochelidae although a cytological demonstration of male haploidy has only been given for members of the Tetranychidae and Tarsonemidae. An investigation by Sokolow (1934) on several species of the group Gamasidae, to which belong the families Laealaptidae and Macrochelidae, showed the existence of two maturation divisions during spermatogenesis and therefore gave strong indication for diploidy of the males of this group. Researches by Ohmori (1936) on the laealaptid mite *Liponassus bacoti*, which have later been confirmed by Filipponi (1955) in the Macrochelidae *Nothrholaspis fimicola*, demonstrated that unfertilized females lay eggs that are able to develop parthenogenetically into male individuals only. Fertilized eggs only develop into females and Filipponi showed that *Nothrholaspis fimicola* lays eggs of a single type and is facultatively parthenogenetic. Arrhenotoky seems therefore to be fairly widespread among Acarina and detailed cytological studies should be made on this group.

Cytological research on arrhenotokous Thysanoptera is wholly lacking and investigations on the chromosome cycles of rotifers have led to contrasting conclusions (Tauson, 1927; Whitney, 1929) although arrhenotokous parthenogenesis has been well ascertained in the complicated life cycle of the rotifers. The problems connected with sex determination in rotifers will be discussed in Chapter 11.

The abovementioned examples show that male haploidy is present in several different phyla where it has evolved independently. Some authors consider it highly improbable that such a coincidence could be completely by chance and put forward a general interpretation of this phenomenon.

Darlington (1939) advanced the idea that arrhenotokous species would have evolved from species where, like in *Icerya purchasi*, a diploid hermaphrodite and an haploid male co-exist. Padoa (1964) remarked on the other hand that arrhenotokous species belong to orders where (as in insects and in arachnids) hermaphroditism is quite exceptional and suggested that arrhenotokous species may have originated from species where parthenogenetic processes normally give rise to diploid females. The appearance of arrhenotokous males in such parthenogenetic strains would convey considerable advantages and Padoa's interpretation fits well with the known facts about cycles where diploid females and haploid males are produced parthenogenetically.

The recent discoveries of Brown and Bennett on the type of male haploidy which is peculiar to Diaspine scale insects confirms, however, the previous hypothesis of Schrader and points to an independent evolution of different methods of sex determination through male haploidy. The current genetic work on the inactivation of one X chromosome in female mammals also lends indirect support to the possibility of inactivating a whole chromosome set in the course of a long evolutionary process.

It appears thus that the known instances of male haploidy have originated in two different ways: (1) through the inactivation of one haploid set and the consequent parthenogenetic development of haploid eggs into males belonging to originally amphigonic groups; (2) through development of haploid eggs into male individuals in groups where female producing parthenogenesis was already widespread.

CHAPTER 11

SEX AND HETEROGONIC CYCLES

CYCLICAL parthenogenesis—which is also named heterogony—is characterized by the alternation of one or more parthenogenetic generations with an amphigonic generation. Some populations may undergo several complete cycles or a single cycle in the year. Other populations produce amphigonic females and males quite exceptionally and some never at all so that acyclical or constant parthenogenesis takes place.

Three main types of sex determinations have been observed in cyclical parthenogenesis: the already described case of arrhenotoky with unfertilized eggs which develop parthenogenetically into males, deuterotoky or amphitoky with eggs which develop into both sexes and thelytoky with unfertilized eggs that develop into female individuals.

Haploid eggs that develop parthenogenetically into males undergo normal meiotic processes, as shown in the previous chapter. Diploid eggs are on the contrary produced in thelytokous and in amphitokous parthenogenesis, and the diploid number of chromosomes is obtained either through secondary mechanisms, which modify the results of the meiotic process as far as chromosome number is concerned, or through the abortion of the meiosis altogether. Meiotic or automictic parthenogenesis must thus be separated from ameiotic (White, 1945) or apomictic (Suomalainen, 1950) parthenogenesis as genetic variability is originated by recombination in the strains that undergo meiotic parthenogenesis and no genetic variability can arise through maturation processes where meiosis has been abolished altogether. It can be assumed that sex variability is correlated to the segregation of multiple sex genotypes in strains showing meiotic parthenogenesis (Bacci, 1957) but such variability of sex genotypes is ruled out within strains where ameiotic parthenogenesis takes place although they may be endowed with a high degree of heterozygosy. It is important therefore to review old and recent cytological work concerning the two types of parthenogenesis.

Sex in Meiotic and Ameiotic Parthenogenesis

The modern work on parthenogenetic eggs started with a remarkable paper by Weissmann (1886) who established that a single polar body is formed in the parthenogenetic egg of *Daphnia* where the second maturation division is apparently abolished and therefore the diploid number of chromosomes is maintained. Similar observations were made by Blochmann (1887) on the parthenogenetic eggs of aphids and soon a number of researches were undertaken in order to verify the validity of Weissmann's generalization. They led to the conclusion that the diploid chromosome number can be obtained in the ripe parthenogenetic egg through a large variety of methods.

1. The Re-establishment of the Diploid Number of Chromosomes in Meiotic Parthenogenesis

Cognetti (1961) has published a diagram in which are shown (Fig. 11.1) the three main methods through which the diploid number of chromosomes can be obtained in meiotic parthenogenesis. Diploidy and haploidy are referred to the chromosomal conditions of the germ line nuclei, as suggested by Suomalainen with reference to the polyploidy of some parthenogenetic species.

Cognetti's classification refers to the time when the processes leading to the diploidy of the ripe egg take place.

Premeiotic regulation takes place, according to Omodeo's observations (1952), in Oligochaeta of the Lumbricid family. In *Eiseniella tetraedra* the chromosomes, which are formed during the last mitotic division prior to the formation of oocytes, remain included within a single cellular membrane during the stage corresponding to the last metaphase. In this way oocytes show at leptotene stage a number of chromosomes which is double the number previously observed in the oogonia. A normal meiotic process takes place and the oogonial chromosome number is obtained again through the extrusion of two polar bodies.

A clear example of the process which Cognetti calls intrameiotic regulation is shown in the tetraploid races of the moth *Solenobia lichenella* (Seiler, 1923). The two anaphase plates formed at the first maturation division unite again to form a single nucleus which has the oogonial number of chromosomes. The second division forms the single polar body which also preserves the chromosome number characterizing the germ line cells.

A different example of intrameiotic regulation has been studied by Narbel Hofstetter (1946) in another psychid moth, *Apterona helix*, which undergoes amphigonic reproduction in the Mediterranean countries but reproduces parthenogenetically in central Europe. The first metaphasic plate of the parthenogenetic egg is formed by thirty-one bivalents but two half-sized spindles are formed through the cutting of the original spindle. These at first lie end to end until they twist around and eventually come close together forming a diploid plate in the spindle of the second division. This division forms two diploid nuclei with sixty-two chromosomes which, however, do not develop into the oocyte and the polar body nucleus but function as the first cleavage nuclei.

The existence of intrameiotic regulation has also been demonstrated in the maturation of the parthenogenetic egg of aphids and of daphnids (Cognetti, 1961; Bacci, Cognetti and Vaccari, 1961) but the process analysed by Cognetti will be discussed in connexion with the known examples of ameiotic parthenogenesis.

Postmeiotic regulation takes place in some of the earliest known examples of automictic parthenogenesis as was observed as early as 1889 by Hertwig who demonstrated that the nucleus of the second polar body fuses with the oocyte nucleus in starfish eggs which develop parthenogenetically.

Postmeiotic regulation shows the closest similarity with processes of self-fertilization. Meiotic parthenogenesis allows therefore a certain degree of genetic variability in the first parthenogenetic generations but, like self-fertilization, rapidly leads to homozygosy. This may have a considerable influence upon the appearance of amphigonic individuals in heterogonic cycles.

2. *Endomeiosis, Transient Chromosome Pairing and True Ameiotic Parthenogenesis*

De Baehr (1920) observed a transient pairing of chromosomes in the parthenogenetic eggs of an aphid which he named *Aphis palmae*. Similar observations were made by other authors in aphids and in other parthenogenetic organisms but such processes were evidently interpreted, from a superficial evolutionary point of view, as the traces of a former maturation through truly meiotic divisions. The species showing the so-called transient pairing of chromosomes were thought therefore to undergo ameiotic parthenogenesis because a single polar body was formed as a result of maturation and a diploid number of chromosomes was found both in the nucleus of the oocyte and of the polar body.

A close re-examination of the whole process both by cytological and by genetical methods (Cognetti, 1961a,b) demonstrated, however, that meiotic processes actually take place in the parthenogenetic eggs of aphids and that such processes are truly functional because they lead to genetic variability within purely parthenogenetic strains.

Formation of bivalents has been observed in the three species of aphids *Macrosiphum rosae*, *Myzodes persicae* and *Brevicoryne brassicae* whose meiotic processes differ only in details (Cognetti, 1961a). The bivalent chromosomes become highly contracted into two pairs of strictly linked hemispheres when the oocyte passes from the germarium to the ovarian chamber. The single bivalents and the univalents remain within the nucleus because no achromatic spindle is formed and the nuclear membrane is not dissolved. The univalents can be observed when some chromosomes are still paired, the detachment being not synchronous. After all the bivalents have separated the chromosomes, whose diploid number has been restored, either concentrate in the centre of the nucleus, as in *Macrosiphum rosae*, remain scattered as in *Brevicoryne brassicae* or tend to approach each other as in *Myzodes persicae*.

Chromosomes then become less distinct and filaments and scattered chromatin granules appear in the nucleus. The oocytes increase considerably in size, big vacuoles appear in the cytoplasm and the nucleus shifts towards one side of the egg cell. The polar kinesis and the formation of the single polar body takes place after the appearance of elongated chromosomes in diploid number which contract considerably when the nuclear membrane has dissolved. This division corresponds therefore to the second maturation division and the single polar body corresponds to the second polar body.

The course of the meiotic process occurring in aphids has been summarized in Fig. 11.1 as an example of intrameiotic regulation of the chromosome number which only differs from the case of tetraploid *Solenobia lichenella* because the first meiotic division takes place within the nuclear membrane, which is not dissolved in aphids as in *Solenobia*.

The functionality of the meiotic process in aphids was demonstrated through selection experiments in a parthenogenetic strain of *Myzodes persicae*, developed from a single female. Selection was successful both against the appearance of winged forms, which disappeared within a few generations, and against the appearance of wingless individuals whose proportion was greatly increased.

Such genetic variability in strains, that were formerly regarded as clones, is easily explained on the basis of genetic recombination and for such reason the meiotic process occurring in the parthenogenetic eggs of aphids can be regarded as perfectly functional and has been named endomeiosis.

The existence of endomeiosis has also been demonstrated in *Daphnia pulex* (Bacci, Cognetti, Vaccari, 1961), belonging to that order of Cladocera that formed the subject of the first researches by Weismann (1876–1879). Endomeiosis in *Daphnia pulex* follows the same course as in aphids: the single polar body discovered by Weismann corresponds to the second polar body, as demonstrated in the case of aphids, and it can safely be assumed that recombination can give rise to genetic variability within single parthenogenetic lines of *Daphnia*, as has been genetically demonstrated in parthenogenetic lines of aphids. This explains also why Banta and Wood (1927) remarked that variability in thelygenous parthenogenesis of daphnids is as high as in the offspring of amphigonic reproduction. Endomeiosis in *Daphnia* shows also the possibility of obtaining different sex genotypes through recombination within parthenogenetic lines of daphnids and consequently the possibility of a not purely phenotypic sex determination.

The recent researches on endomeiosis in aphids and daphnids pose on the other hand the problem whether the so-called transient chromosome pairing observed in other instances of ameiotic parthenogenesis actually implies cross-over between the homologous chromosomes and chiasma formation, although chiasmata would not be necessary to demonstrate cross-over. The observations of Seiler (1947) on a rudimentary reduction division in parthenogenetic eggs of the curculionid beetle *Otiorrhynchus sulcatus* are very interesting in this respect and so are the observations on chromosome pairing made by Artom (1931) on specimens of *Artemia salina* belonging to a diploid parthenogenetic population from Sete. Similar observations were also made by Belar (1923) on *Rhabditis monohystera*, a nematode, whose parthenogenetic eggs produce a single polar body.

There are many other similar cases and it is sufficient to conclude that the

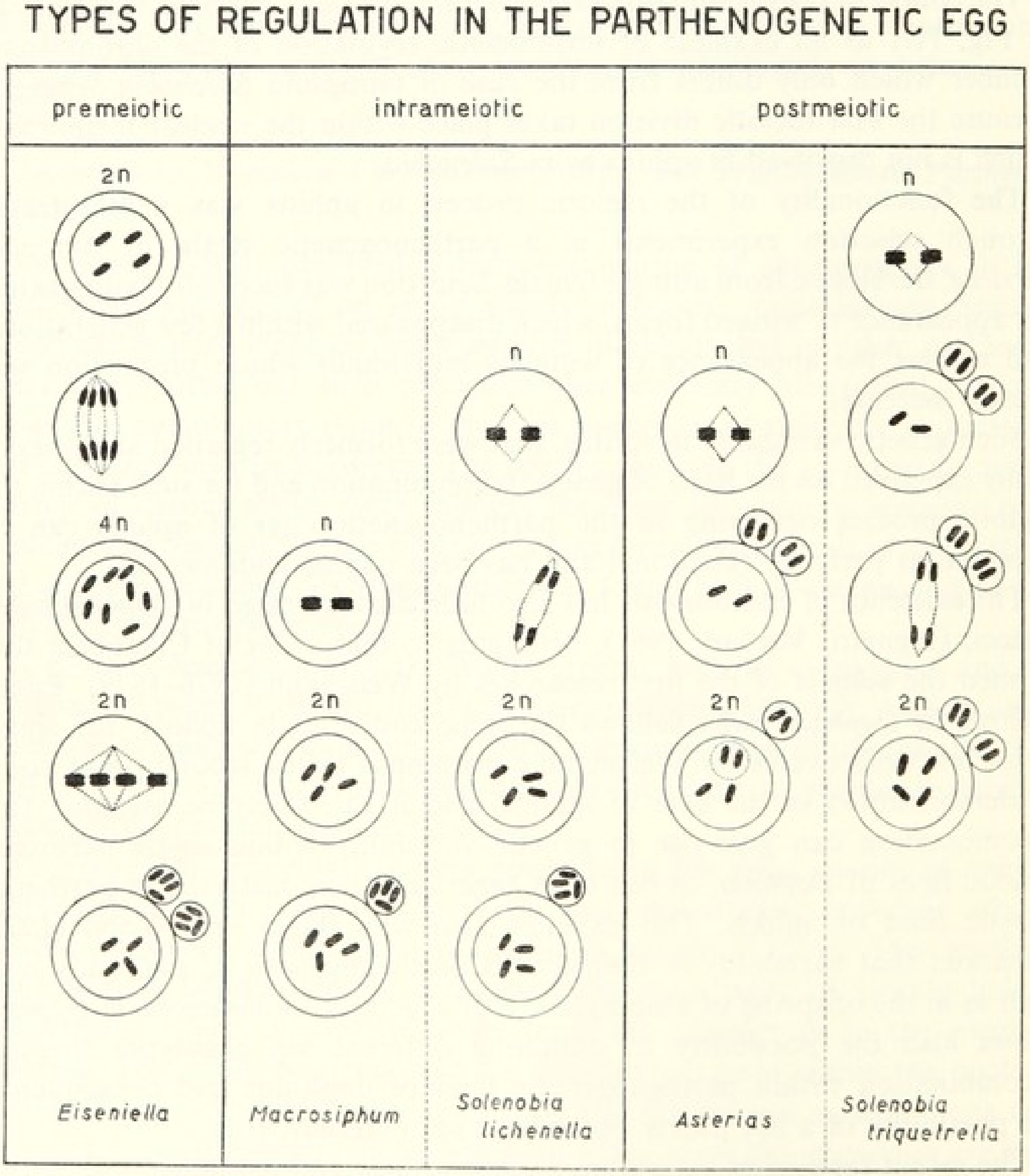


FIG. 11.1. The chromosome number is regulated in meiotic parthenogenesis by processes which take place prior, during or after the meiotic process (Cognetti, 1961).

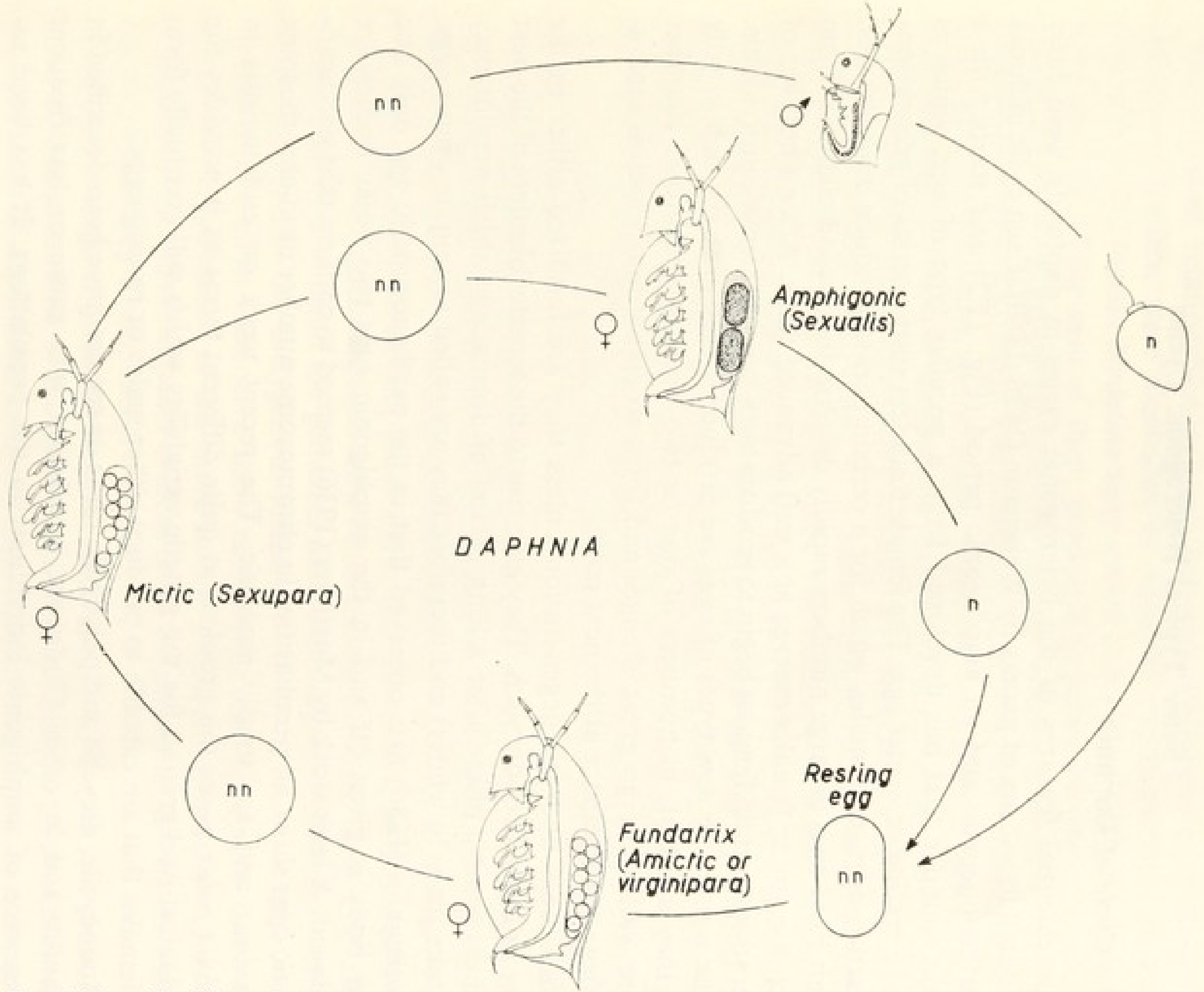


FIG. 11.2. The life cycle of *Daphnia*, an example of cyclical diploid parthenogenesis. The generations of amictic females, that are often observed before mictic females appear, have been omitted.

problem of the existence of ameiotic parthenogenesis deserves a closer examination. Before approaching the problem of sex determination in thelygenous and amphogenous parthenogenesis it is convenient to examine some specific examples of heterogonic cycles in different groups.

Some Typical Heterogonic Cycles

1. The Cycle of Daphnia

The essential features of the heterogonic cycles in daphnids were early elucidated in a series of papers by Weismann (1876–1880). Each cycle includes a parthenogenetic and an amphigonic period (Fig. 11.2) and starts with a female founder that has developed from a peculiar type of egg, which is called a resting or winter egg. The female founders start a series of generations of parthenogenetic females which for a certain period produce only females. Unfertilized eggs in large numbers rapidly develop in a brood chamber from which the young females emerge at a well advanced stage. After a number of generations certain females begin to produce both male and female individuals. At the same time new types of eggs are produced; they are very rich in yolk and they are laid, in numbers of one or two only, in the brood chamber whose walls undergo great changes and form an ephippium which is separated from the carapace at the next molt.

These eggs undergo degeneration unless they are fertilized either in the ovary or in the brood pouch. They give rise to the female founders of the next parthenogenetic phase after a long period of dormancy (which may or may not take place in winter) and therefore they are called resting or winter eggs.

Weismann (1886) first observed that in the parthenogenetic egg only one polar body is given off and in the amphigonic eggs two polar bodies are produced. A later work by Mortimer (1936) seemed to confirm that parthenogenetic eggs show no peculiarities in chromosome number or in chromosome behaviour and are totally ameiotic. The recent work on endomeiosis in *Daphnia pulex* poses the problem in quite different terms as it provides the cytological background for the genetic variability which will eventually form the females that are capable to produce either males or resting eggs.

Monocyclic, dicyclic and polycyclic races or species have been described in daphnids and in other Cladocera in relation with more or less frequent appearance of amphigonic individuals in the populations. It has been remarked that in limnetic populations of large lakes, reproduction may be entirely parthenogenetic or acyclic and in smaller lakes and ponds several complete cycles may be observed during the year. Resting eggs have been discovered in Arctic lakes in the absence of males and of amphigonic reproduction (Edmonson, 1955).

The idea of the existence of inherent genetical or physiological rhythms which are responsible for the occurrence of amphigonic forms will be discussed later with a brief survey of experimental work on the determination of sex.

2. *Mictic and Amictic Females in the Cycles of* Hydatina *and* Asplancha

The genetical relationships existing between reproductive categories of heterogonic rotifers, were illustrated by Maupas' experiments in *Hydatina senta* (1891): 342 female *Hydatina* were mated to a corresponding number of males and the 74 per cent of them produced fertilized resting eggs, the remaining 26 per cent produced unfertilized eggs which immediately gave rise to females. A second group of 480 female *Hydatina* was left unmated and the 75 per cent of them gave rise to males, while the remaining 27 per cent produced females. The results obtained in mated and unmated groups of females showed that both males producing females and females that are capable of producing fertilized eggs belong to a same category which is quite distinct from the category of thelytokous females. The existence of the two categories of females was later confirmed by Whitney (1913) in his work on *Asplanchna*.

The females that produce males when they are not fertilized or resting eggs when they are fertilized were called mictic females by Storch (1924) and they correspond to the so-called sexuparous females (Caullery, 1913). Females that reproduce only parthenogenetically and give origin to female individuals only were called amictic females by Storch and virginoparous females by Caullery (Plate I).

No morphological difference between mictic and amictic females has been discovered among rotifers but the distinction is a fundamental one from a general point of view. It clearly demonstrates the existence of different sex genotypes among the uniform phenotypes that at certain periods compose a population of parthenogenetically reproducing females. It is important therefore to have in mind the genetical difference demonstrated between the arrhenogenous and the thelygenous females.

Rotifers as well as Cladocera show monocyclic and polycyclic races and the adaptive significance of the more or less frequent appearance of males during the annual cycle appears evident from a number of ecological observations (Wesenberg Lund, 1930).

From a cytological point of view the situation in rotifers is not clear yet. The only genus studied is the genus *Asplanchna*: Tauson (1924–1927) said that both sexes of *Asplanchna intermedia* are diploid with twenty-four chromosomes. Whitney (1929) however found twenty-six chromosomes in the female of *Asplanchna amphora* and only thirteen chromosomes in the males. Two kinds of sperms are formed in this species: large motile ones and small ones that do not function in fertilization.

The whole question of the chromosome cycle is extremely uncertain at present and it needs a careful re-investigation.

It must also be pointed out that Ruttner demonstrated (1946) that the planktonic rotifer *Anuraea aculeata* (*Keratella quadrata*) may produce eggs having the character or resting or "winter" eggs which, however, have not been fertilized.

External factors play an important role in the determination of mictic females and of male individuals but there is ample evidence for the existence of genetic variability also in the heterogonic cycles of rotifers.

3. The Cycle of Neuroterus

Neuroterus lenticularis is the representative of the Cynipidae family of Hymenoptera whose cycle has best been studied.

Cynipidae typically show two generations a year, one parthenogenetic, the other amphigonic and in *Neuroterus* winter eggs are found on lenticular galls at the inferior face of leaves in *Quercus,* in Subitaneous eggs are laid on germs of the same plant where small galls are found. Researches by Doncaster (1910–1916) and Dodds (1939) showed that two types of parthenogenetic females exist which although they are phenotypically alike, differ in being either female or male producers: they are therefore thelygenous and arrhenogenous females respectively. In the eggs of both types the chromosome form ten bivalents during oogenesis. In arrhenogenous females a normal meiosis takes place and in the eggs of thelytokous females no meiosis seem to take place and no polar bodies are produced. Haploid and diploid eggs are thus formed which, according to the general rule, give rise to male and female individuals respectively (Fig. 11.4).

Oogenesis is normal in the amphigonic generation, spermatogenesis is essentially the same as in all haploid males. The winter fertilized eggs, which all contain twenty chromosomes are nevertheless sharply differentiated in two categories which correspond to male and female producing lines.

The results of work on *Habrobracon* indicate a reasonable explanation in the absence of any cytologically appreciable difference between the two lines. The female producing line is probably heterozygous for one or more genes, the male producing line is homozygous for the same genes.

Chromosomal formulae may thus be the following: ♂ = n, ♀ = n n' where each haploid set corresponds to ten chromosomes.

The case of *Neuroterus* provides extra evidence that strongly differentiated sexual lines can be obtained even in the absence of visibly differentiated sex chromosomes.

4. Chromosome Cycle and Environmental Conditions in Heteropeza pygmaea (Oligarces paradoxus)

Heteropeza pygmaea is a fungus feeder which belongs to the family Cecidomyidae and its life cycle has been described by Uhlrich and Camerzind (1936, 1940, 1962).

Heteropeza reproduces by larval parthenogenesis (also called paedogenesis) and by amphigony although most species of Cecidomiidae are normally bisexual. The larvae that develop from eggs which have been laid by adult females are called the mother larvae and produce only female larvae. Such "daughter" larvae are characterized by an X-shaped eye-like structure and by the absence of the sternal spatula which is typical of most cecidomiid larvae. The daughter larvae are able by paedogenesis to develop progeny of four different types:

1. Arrhenogenous larvae that will produce by paedogenesis male larvae from which adult males will develop.
2. Amphogenous larvae from which both male larvae and "daughter" type larvae will be produced.
3. Thelytokous larvae which will develop into "daughter" larvae only.
4. Female larvae which develop into adult female gnats (Camerzind, 1962).

Eggs laid from mated and unmated females differ very much in the number of mother larvae which develop from them, 63 per cent of larvae from mated females transforming into fertile mother larvae and only 5 per cent of larvae from unmated females giving rise to such mother larvae of the first generation. The marked differences in the vitality of larvae from mated and from unmated females has been explained by assuming that the former develop from amphigonic, the latter from parthenogenetic eggs.

Camerzind established that the first generation larvae (the mother larvae) are always females and are only capable of paedogenetic production of the female daughter larvae who are able on the contrary to originate different types of progeny.

The sex and the type of progeny of the daughter larvae is basically determined, according to Uhlrich (1934, 1936), by the influence of the nutrition and, given a certain quality of food, by the food quantity. Research by Nikolei (1961) on different fungus feeding species showed also an influence of the food quality—that is of the fungus species—and an influence of the temperature conditions. The latter appear to influence the larvae directly and not only indirectly through influence on the growth on the fungal hyphae that are used as food in the cultures.

The chromosome cycle of *Heteropeza pygmaea* has been investigated by Reitberger (1940) and by Hausschteck (1962) and shows essentially the same differential elimination of chromosomes from somatic nuclei and the same regulative processes that have been extensively investigated by White (1950) in other species of paedogenetic Caecydomiidae. The similarity with chromosome cycles occurring among the Sciaridae (see Chapter 4) is striking and seems to be based on a fairly close relationship between the two families.

Male eggs are bigger than female eggs of the same stage and they can be distinguished before the first maturation division in the paedogenetic larvae. The male eggs undergo both maturation divisions and their chromosome number is consequently reduced to thirty-eight or thirty-nine chromosomes. The smaller female producing eggs retain a number of chromosomes which is close to seventy-seven because they undergo a single equational maturation division. Four polar bodies, that will participate in the segmentation processes, are formed during the maturation of the male eggs and a single polar body—that soon degenerates—is formed during the maturation of the female eggs.

A regulation of the chromosome number of the male eggs is carried on through the fusion of two or more somatic nuclei of the mother with the egg nucleus. The germ line nuclei of the male has a high number of chromosomes which, however, is not the same as the chromosome number prior to meiosis.

Chromosome eliminations take place between the second and the fourth segmentation division and between the seventh and the eighth division. The somatic line nuclei of the male and of the female embryos present five and ten chromosomes respectively at the end of such processes so that a mechanism of somatic haplo-diploidy regulates the second step of sex determination after the destiny of the eggs has been determined in early stages by environmental influences upon the daughter larvae. The absence of any indication concerning variability within different strains hinders any consideration regarding the possible existence of multiple sex genotypes in *Heteropeza pygmaea* and in related species.

4. The Cycle of Phylloxera caryaecaulis

The existence of two well distinct male producing and female producing lines has been established in *Phylloxera caryaecaulis*, a species of aphid whose cycle consists, according to Morgan (1909–1915), in only three generations.

The females born from the fertilized resting eggs are known as fundatrices (Fig. 11.3) and, although they are morphologically identical, they are certainly divided into two classes. One class forms thelygenous females: the other class forms arrhenogenous females. The arrhenotokous or thelytokous females of the second generation are generally known as sexuparous females or sexuparae because they generate male and female individuals from two kind of eggs. The male and female individuals, that were generated by parthenogenesis, are usually called sexuales. They pair and from the eggs laid by the fertilized females emerge the fundatrices of the next year.

All the different types of females have a diploid set of eight chromosomes consisting in two pairs of autosomes and in two pairs of X chromosomes. Males have six chromosomes with two pairs of autosomes and one pair of X chromosomes only. The eggs of both fundatrices and thelytokous females undergo a single maturation division, which is non-reductional. The small

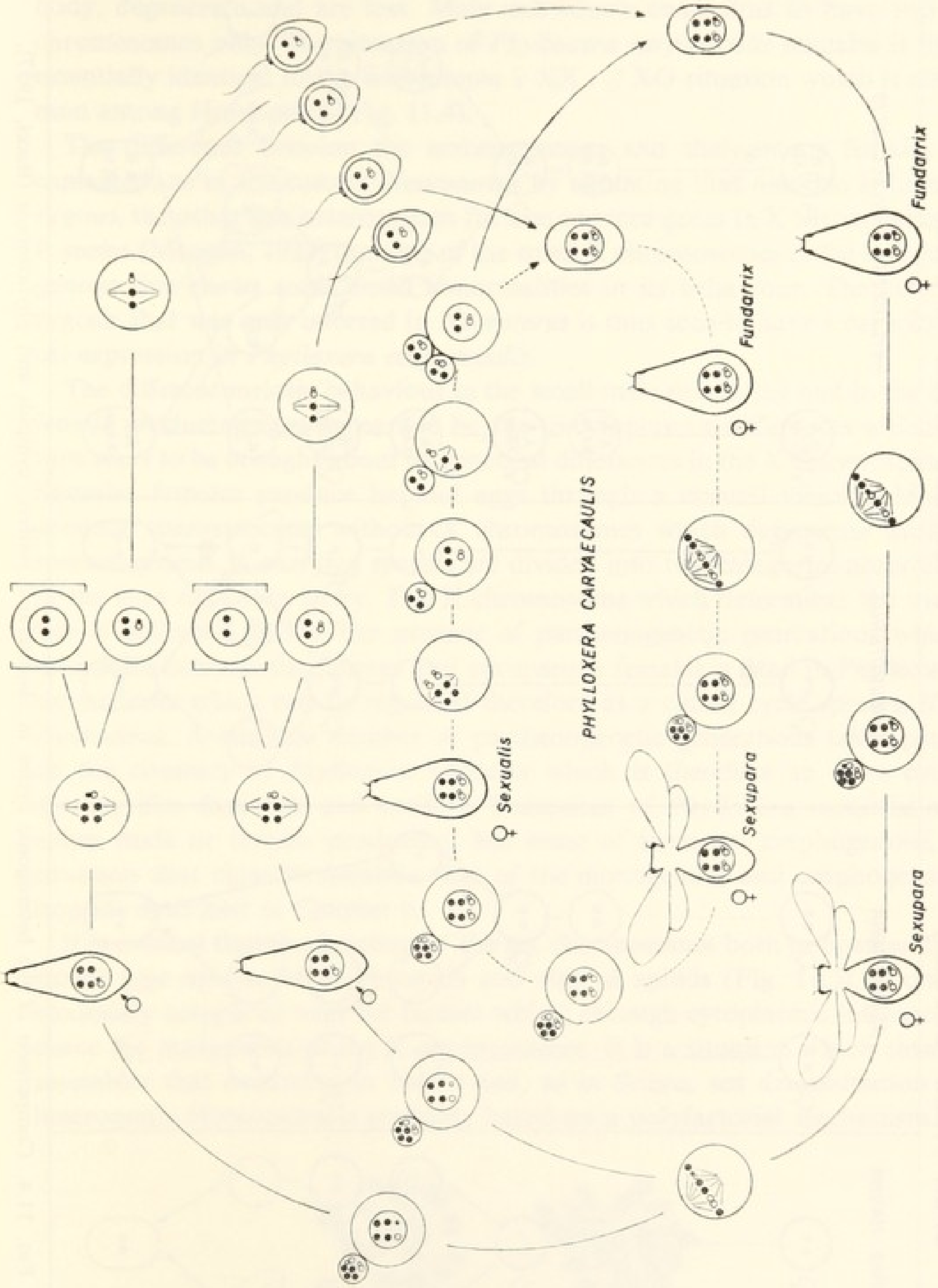


FIG. 11.3. The life cycle of *Phylloxera caryaecaulis* (after Morgan, 1912–1915).

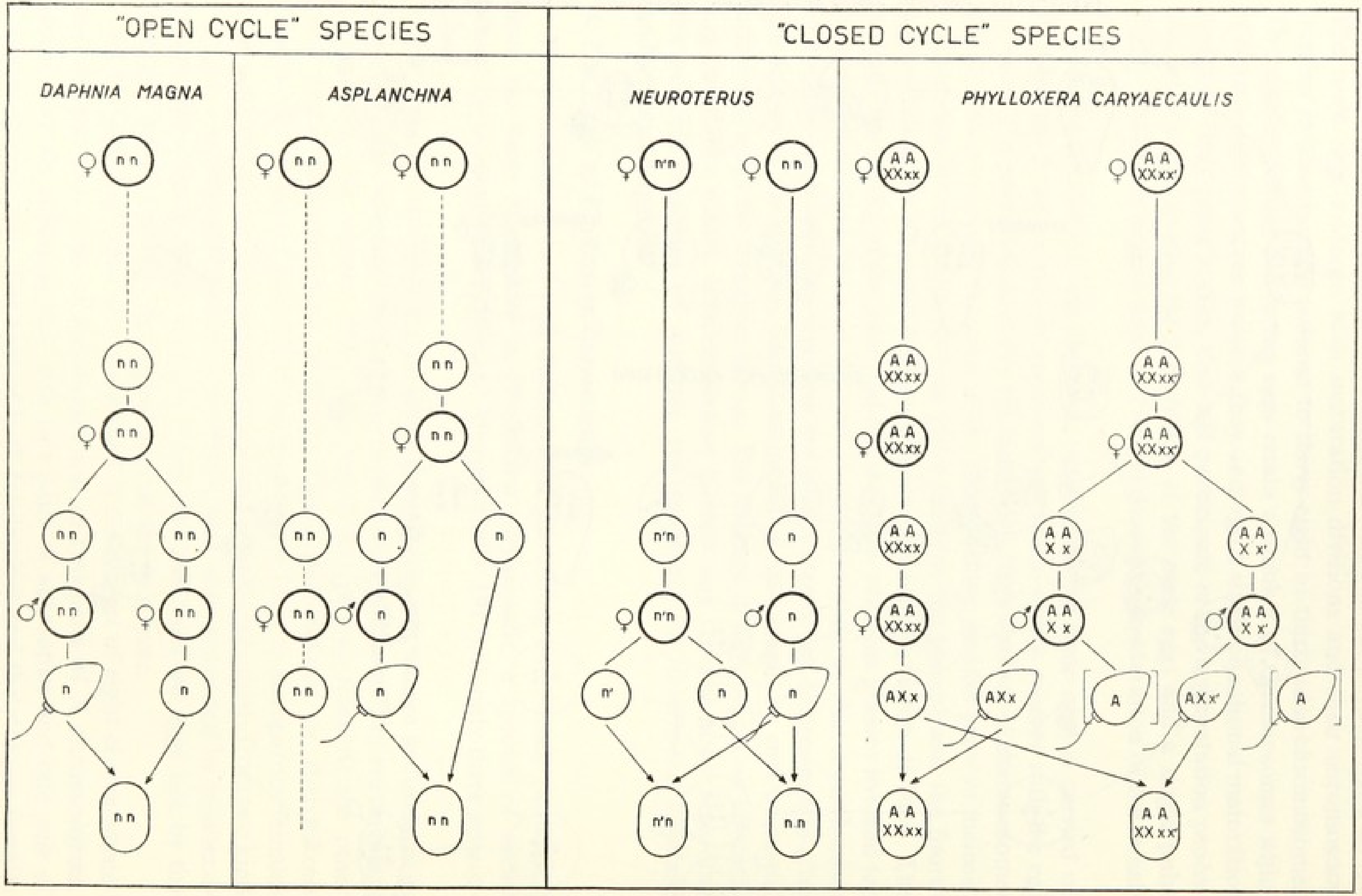


FIG. 11.4. Comparison of the heterogonic cycles in cladocerans, rotifers, cynipids and phylloxerans (Bacci, 1957).

eggs of arrhenogenous females also undergo a single division but two X chromosomes remain behind in the spindle during the formation of the polar body, degenerate and are lost. Male individuals come thus to have two X chromosomes only. The situation of *Phylloxera caryaecaulis* sexuales is thus essentially identical to the well-known ♀ XX : ♂ XO situation which is common among Homoptera (Fig. 11.4).

The difference between the arrhenogenous and thelygenous females is explained, as in the case of *Neuroterus*, by assuming that one line is homozygous, the other line heterozygous for one or more genes in X chromosomes. It seems (Morgan, 1923) that one of the small X chromosomes in the arrhenogenous line shows some small abnormalities in its behaviour. The heterozygosis that was only inferred in *Neuroterus* is thus seen to have a caryological expression in *Phylloxera caryaecaulis*.

The different meiotic behaviour in the small male producing and in the big female producing eggs appears to be due to cytoplasmic differences which in turn seem to be brought about by genetical differences in the X chromosomes. Sexuales females produce haploid eggs through a normal meiosis. Males produce spermatocytes without X chromosomes which degenerate during spermiogenesis. X-carrying sperms are divided into two categories according to the type of X they carry. The X chromosome which determines the male line is indicated as X'. The number of parthenogenetic generations which take place between fundatrices and sexuparous females is fixed in *Phylloxera caryaecaulis* which can be regarded therefore as a closed cycle species, like *Neuroterus*. A variable number of parthenogenetic generations takes place on the contrary in *Phylloxera vastatrix* which is therefore an open cycle species, like daphnids and rotifers. Sexuparae of *Phylloxera vastatrix* are either male or female producing, but some of them are amphogenous, a situation that closely resembles that of the monogenous and amphogenous isopods described in Chapter 6.

It is evident that the function of the sex chromosomes both in open and in closed type species of phylloxerids and also of aphids (Fig. 11.5), is quite secondary compared with the factors which, through cytoplasmic influences, cause the movements of the X chromosomes. It is a situation which closely resembles that occurring in *Sciara* and, as in *Sciara*, sex determination of heterogonic Homoptera is probably based on a polyfactorial mechanism.

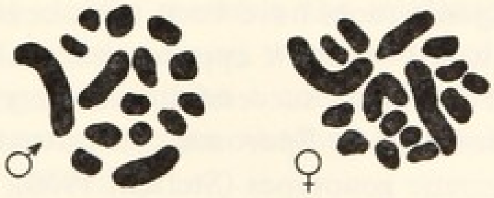


FIG. 11.5. Spermatogonial and oogonial metaphases of *Brevicoryne brassicae*, an aphid showing cyclical diploid parthenogenesis (courtesy of G. Cognetti).

A more detailed study of sex variability and of sex ratios is needed therefore in order to identify the multiple factors of sex determination. The distribution of different kinds of parthenogenetic races and species is at present the best investigated aspect of sex variability in heterogonic cycles.

6. *Parthenogenetic Races and Sex Variability*

The small branchiopod crustacean *Artemia salina* has a diploid amphigonic race and different parthenogenetic races which are diploid with 2 n = 42, tetraploid with eighty-four chromosomes (Artom, 1931) and even pentaploid or octoploid (Barigozzi, 1957). Research on this species, which has a very dispersed area of distribution owing to its habitat in salt lakes, has concentrated mainly in the determination of the grade of ploidy and in the study of the maturation of the parthenogenetic egg but very little information is available regarding the factors determining the appearance of exceptional males in the diploid and tetraploid parthenogenetic races. No precise data on the sex ratios of the diploid amphigonic populations were available until Bowen (1963) found a white partially sex-linked gene in a stock derived from crosses of the Utah and the California races. The development of such research on the formal genetics of *Artemia* will provide the basis for the interpretation of different genetic mechanisms that characterize the sex situations in the races having different genomic constitutions.

Haploembia solieri belongs to the small insect order of Embioptera and is represented by an amphigonic race living in Italy, France, Spain and the Balkans, and by a parthenogenetic race which is isolated in Sardinia and in Corsica and lives mixed with the parthenogenetic race in the Tuscan archipelago. Stefani (1959) established that the male of the amphigonic race has a set of nineteen chromosomes and the females have twenty chromosomes. The sex chromosome mechanism is thus of the *Protenor* type. The parthenogenetic individuals have twenty-two chromosomes and Stefani interprets the situation of the parthenogenetic strains as an example of tetrasomy of the X chromosome (18 A + XXXX). Females with thirty-three chromosomes are also found among the parthenogenetic individuals. No synapsis of homologous chromosomes has been observed in the parthenogenetic eggs, a condition which evidently favours the formation of triploid individuals.

No males have ever been observed among thousands of individuals from parthenogenetic strains and attempts to mate parthenogenetic females with males from the amphigonic races have been unsuccessful. Sex ratios in the amphigonic populations is 1 : 1 but it appears that males which are actually capable of mating and fertilizing successfully are very scarce because most of them are heavily parasitized by Sporozoa. This situation would favour the diffusion of parthenogenetic genotypes (Stefani, 1960).

It appears that, from the point of view of sex determination, the parthenogenetic tetrasomic strains of *Haploembia* show but one genotype so that a true kind of sex monogamety is established in coincidence with the absence of sex variability.

A similar situation seems to have been established in parthenogenetic oligochaete worms which, however, develop from strains where hermaphroditism is well established.

Eiseniella tetraedra typica is a common earthworm which shows a triploid parthenogenetic mutant ($3\,n = 54$, Fig. 11.6) and a tetraploid mutant ($4\,n = 74$). The former is most widespread in Italy (Omodeo, 1952), the latter is widespread in England (Muldal, 1952) and has been found in mountains of southern Italy and of Turkey (Omodeo, 1955).

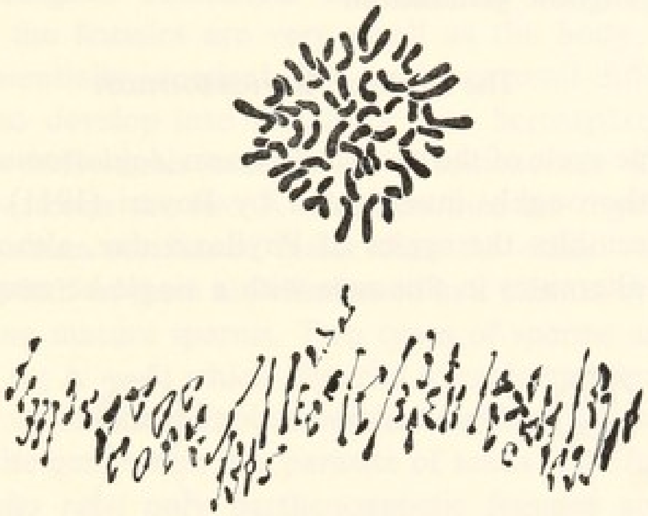


FIG. 11.6. Ovogonial metaphase (a) and first metaphase of the egg (b) of the triploid strain of *Eiseniella tetraedra*, an obligatorily telytokous earthworm (from Omodeo, 1952).

Abortive spermatogenesis and empty spermathecae have been observed in individuals of the different strains which therefore present constant parthenogenesis, and apparently single sex genotype and sex monogamety.

Observations by Marchal (1913) on the species *Chermes piceae*, which lives in France on *Abies pectinata*, have provided some information regarding the disappearance of males and of the amphigonic reproduction in a species that originally showed heterogonic cycles. Reproduction takes place on *Abies pectinata* and is exclusively parthenogenetic in the absence of any male individuals. Repeated attempts to obtain amphigonic reproduction upon two species of *Picea* completely failed and no males were produced although winged individuals that closely resemble the sexuparous females of a related species (*Chermes nordmannianae*) were generated by *Chermes piceae*. Marchal regarded *Chermes piceae* as a constantly parthenogenetic species which has evolved from a species showing cyclical parthenogenesis by reduction of its sexual variability.

Similar observations were made by Banta (1925) on strains of the cladoceran *Moina macrocopa*, which, after having reproduced exclusively by parthenogenesis for several generations, nearly lost their ability to produce males under laboratory conditions.

Similar results were also obtained on different species of rotifers by Whitney (1912), by Luntz (1929) and were confirmed by Buchner (1941).

Both nature and laboratory observations demonstrated the possibility of reducing sex variability in heterogonic strains both under natural and experimental conditions.

Before discussing the meaning of sex variability in heterogonic cycles it is worth while to examine a cycle in which a gonochoric generation alternates with a hermaphrodite generation which has the chromosome set of the female of the amphigonic generation.

The Cycle of *Angiostomum*

The chromosome cycle of the nematode worm *Angiostomum nigrovenosum*, which has been thoroughly investigated by Boveri (1911) and by Schleip (1911), closely resembles the cycles of Phylloxeridae, although the amphigonic generation alternates in this case with a single hermaphrodite generation.

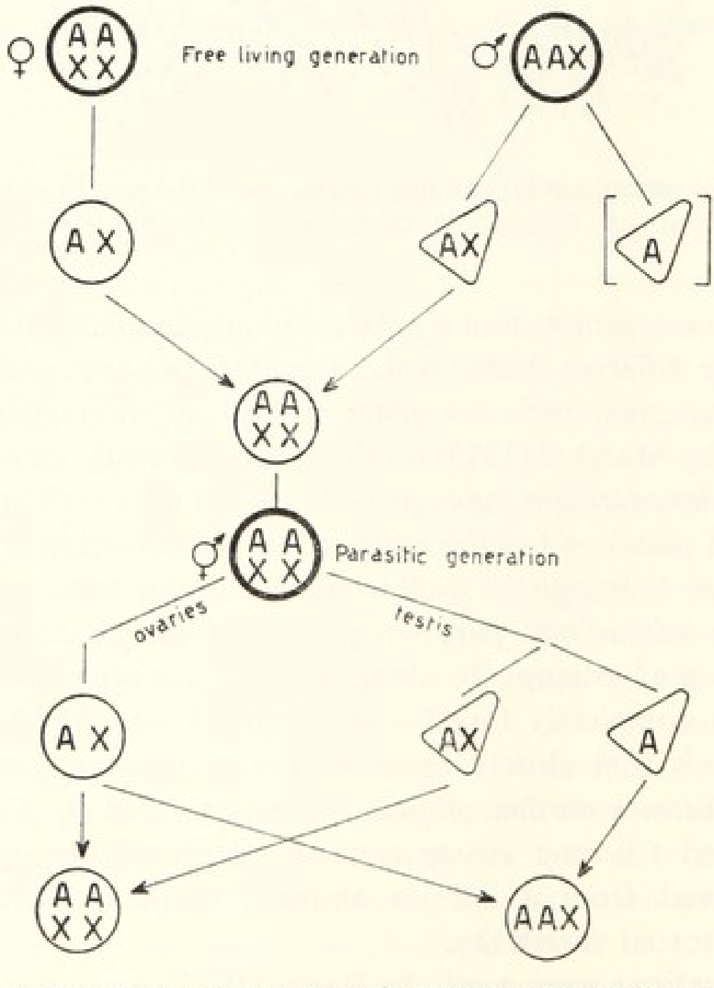


FIG. 11.7. The life cycle of the parasitic nematode *Angiostomum nigrovenosum* (after Boveri, 1911, and Schleip, 1911).

A generation which is parasitic in the lung of the frog is hermaphrodite. The fertilized eggs give rise to a free gonochoric generation and its eggs give rise to the hermaphrodite parasites.

The females of the free living generation are AA + XX = 10 + 2 and the free living males are AA + X = 10 + 1. Females regularly produce eggs with six chromosomes, males produce two types of sperms, with six and with five chromosomes respectively, but the spermatids lacking X always degenerate after a regular meiosis. The remaining spermatozoa show the same chromosome set as the eggs: A + X = 5 + 1. The next parasitic generation contains twelve chromosomes (AA + XX) like the free living female individuals. Morphological differences between the hermaphrodite parasitic individuals and the females are very small as the body and the gonadal structures are essentially identical. The environmental differences cause the same genotype to develop into female or into hermaphrodite phenotypes. The eggs of the individuals contain six chromosomes. During spermatogenesis in the ovotestes the two X chromosomes are regularly divided and one of them becomes condensed faster than the other. All the spermatids contain an X but half of them are eliminated in a plasma bridge which unites the two remaining mature sperms. Two types of sperms are formed in this way (A + X = 6 ; A = 5) which give rise to male and female zygotes.

A very similar cycle has been observed in *Rhabdias fülleborni*, which during the hermaphrodite generation is a parasite of toads (Dreifus, 1937).

In *Strongyloides ratti* only parthenogenetic females are present in the parasitic generations and both male and female individuals are found, in varying sex proportions, in the free living generation. Parasitic and free living females have six chromosomes but males appear to have only five chromosomes (Nigon and Roman, 1952) a condition closely resembling that of *Angiostomum*.

The peculiarity of the cycles of *Angiostomum* and of *Rhabdias* lies in the substitution of hermaphrodites, which are capable of self-fertilization to parthenogenetic females. The transformation of individuals with the chromosome set of the females into hermaphrodites is apparently due to something in the parasitic condition which enhances the male potencies of the female genotypes.

The Factors of Sex Variability in Cyclical Parthenogenesis

The problem of the factors determining sex variability in heterogonic cycles was first posed by Weismann (1880) at the conclusion of his pioneer work on daphnids. He affirmed that the appearance of males after a series of parthenogenetic generations is not due to the direct influence of external factors and that the cycles—which are different in different species or races—are fixed in each of them by hereditary factors. The classification proposed by

Weismann of monocyclic, dicyclic, polycyclic and acyclic populations was found to be valid for rotifers (Lauterborn, 1898) but it was soon noticed that cycles of cladocerans, rotifers and of many aphids are often very irregular and that they are easily influenced by a number of external factors. The analysis of such factors has been the object of much diligent work. Such researches did not lead, however, to very clear conclusions as it was generally supposed that the evidence that the reproductive cycle and the sex variations are strongly influenced by the environment ruled out the possibility of a concomitant genetic control.

1. Evidence for the Environmental Factors

The investigators were guided in the search for specific environmental factors influencing the appearance of mictic (or sexuparous) females and of males by the study of the conditions which prevail in the seasons when such individuals appear in nature.

The work of Maupas (1891) on the rotifer *Hydatina senta* showed that at high temperatures one obtains 97 per cent of amphigonic forms, at low temperature only 24 per cent. The results of other work on the influence of temperature in rotifers were, however, contradictory, with Shull (1911) obtaining more amphigonic individuals at 10°C than at 24°C and other authors being unable to find effect of temperature at all. The appearance of amphigonic forms was also obtained by Mortimer (1936) in some species of Cladocera through temperature changes, that is with both high and low temperatures. The production of winged and amphigonic individuals is also favoured by temperature changes in parthenogenetic lines of aphids (Shull, 1929).

The importance of "change" in environmental conditions rather than of any particular factor has been emphasized by Buchner in his work on several species of rotifers (1941, 1961).

The type of food, its absence or abundance, also strongly influences the change from parthenogenetic to amphigonic (or bisexual) reproduction. Deficiency of food caused the appearance of males in species of Cladocera in Mortimer's experiments (1936), a result comparable to those obtained by Hartmann and Huth (1936) in *Ophryotrocha*. Von Dehn (1937) confirmed such conclusions in *Moina rectirostris* and she started a series of experiments where she used yeast and yeast extracts as food. Cultures which were fed upon whole yeast produced about 30 per cent male individuals and cultures which were maintained on yeast whose fat content had been eliminated produced all female progeny with only a few exceptional males. The addition of the fatty extract to the treated yeast raised the production of male individuals to the original percentages. It was also demonstrated that a mixture of ergosterin and olive oil in 1:1 ratio has the same effect of the fatty extract of yeast and that the formation of winter eggs depends upon the presence of ergosterin in the food (von Dehn, 1955). Von Dehn employed in the course of her experiments the parthenogenetic progeny of a single female with the aim of working on genetically homogeneous material, a condition which, however, appears now very doubtful after the demonstration of endomeiosis in *Daphnia pulex* (see p. 217).

Fries (1964) worked on a strain of *Daphnia magna*, which developed from a single parthenogenetic female, and established the influence of temperature variations in determining the appearance of amphigonic individuals.

Among the great number of researches concerning the influence of food in the cycle of rotifers, it may be mentioned that Whitney (1916) observed an increase in the proportions of mictic females in cultures fed with *Chlamydomonas*, a result that has been confirmed by Gaudenzi and Zunarelli (1960) but not by Pourriot (1957).

Crowding appears also to be an important factor in causing the appearance of mictic females and of males in cyclical parthenogenesis. It has not been established whether this factor acts in limnetic forms through the food shortage, the excess of excretions or the excess of bacteria. Experiments by Treillard (1925) on *Daphnia magna* showed that such species reproduces only by parthenogenesis when kept in an aseptic medium and immediately produces mictic females and males when transferred into a medium where amphigonic individuals had been produced by a different strain in the presence of bacteria. Treillard referred to bacterial factors influencing sex variability and it is possible that external metabolites have indeed the function of ectocrine substances (Lucas, 1947); substances whose importance is being increasingly felt in ecological research.

A so-called "group effect" is also responsible, according to the extensive experiments by Bonnemaison (1951), for the high production of winged sexuparous females in the aphids *Brevicoryne brassicae* and *Myzodes persicae*. The appearance of amphigonic individuals is also favoured in aphids by the reduction of the diurnal phase.

A close adaptation to the natural cycles of environmental condition is made apparent by the multitude of investigations concerning the effect of external factors upon the appearance of amphigonic individuals (and therefore of sex variability) in heterogonic irregular, or open-type cycles. The results of such works are important as the first approach to the evaluation of the complicated mechanisms of sex determination.

Hartmann (1956) accepted von Dehn's conclusions regarding *Moina rectirostris* that all parthenogenetic eggs are primarily determined as females with F factors strongly outweighing the M factors. Special conditions, which in the case of *Moina* consist in the assumed presence of special substances with the food, induce an activation of the M factors whose reaction curve (see p. 185) rises rapidly and overcomes the F reaction curve at an early period. This interpretation appears quite reasonable as a provisory explanation of the interaction between genic sex factors and environmental influences in the sex differentiation of single individuals. It must be pointed out, however, that the above experiments, as all experiments of this kind, provide no proof whatever for the existence of a single sex genotype in parthenogenetic populations having an irregular or open type of cycle.

The existence of parthenogenetic races, with different patterns of sex variability, the known instances of reduced sexual variability both in natural and in laboratory populations, the existence of endomeiosis in the parthenogenetic reproduction of aphids and of *Daphnia* are strong indication that environmental factors do not always exert their influence upon a single sex genotype, as assumed according to the theory of phenotypic sex determination.

2. *The Evidence for Multiple Sex Genotypes*

Several authors have demonstrated that parthenogenetic reproduction can be maintained for an indefinite number of generations if favourable environmental conditions are preserved. Such results were regarded as a proof of the complete control of environmental factors upon the sex cycle and records mentioned as many as 767 generations of purely parthenogenetic reproduction, which were obtained in *Daphnia pulex* by Banta and Brown (cited by von Dehn, 1937), and 546 generations of constant parthenogenesis in the rotifer *Hydatina senta* (Whitney, 1912). Constant parthenogenetic reproduction was also observed by Luntz (1929) in a culture of another rotifer, *Brachionus bakeri*. Purely parthenogenetic strains of *Brevicoryne brassicae* and of *Myzodes persicae* have also been obtained in laboratory conditions (Bonnemaison, 1951).

Numerous observations in nature have also demonstrated that species of aphids, which normally produce sexuparous individuals in cold and temperate climates, show constant parthenogenesis in tropical or subtropical regions.

The above experiments and observations show that some environmental factors favour the expression of the female parthenogenetic phenotype but they give little information regarding the sex genotypes in the parthenogenetic strains. It appears, on the other hand, extremely significant that the strains which have undergone parthenogenetic reproduction for a great number of generations show a reduced capacity to produce males when subjected to factors which formerly induced the reproduction of amphigonic individuals. The irreversible elimination of one sex from formerly cyclical strains has been conclusively demonstrated by the previously cited Marchal's observations on *Chermes*, by repeated observations on strains of aphids in tropical and temperate regions, by the observations of Whitney (1912) and of Luntz (1929) on parthenogenetic strains of rotifers. Such elimination can be regarded as a strong indication of the genetical nature of the sex variability existing prior to the evolution into acyclical strains from mono or polycyclic strains and it could not be explained if the existence of a single sex genotype were assumed for the original strains.

Several authors have concluded (Luntz, 1926; Shull, 1929; Vandel, 1931) that, even if the original Weismann's interpretation can no longer be fully accepted, each heterogonic population shows an inherent type of cycle, which follows a peculiar pattern of sex variability. Vandel expressed this concept by writing, following Caullery (1913), that the cycles of Cladocera and of rotifers must be considered as the results of a specific and hereditary reaction toward external conditions.

3. A Working Hypothesis

The problem early posed by Weismann can thus be postulated in a new way. Does the specific and hereditary reaction, which has been shown by modern authors, depend on the norm of reaction of a sex genotype, which remains single in each heterogonic population, or does it depend on the existence of multiple sex genotypes showing different reaction norms toward the varying environmental conditions and their selective pressure during the annual seasonal cycle?

A working hypothesis has been advanced (Bacci, 1957) to explain certain peculiarities of species that show irregular heterogonic cycles and their possible differentiation into polycyclic, monocyclic or acyclic races. It is assumed that the reactions of the open cycle populations toward the varying conditions of the annual cycle are actually due to the presence of multiple sex genotypes. It has been shown in *Neuroterus lenticularis* and in *Phylloxera caryaecaulis*, two closed cyclic species, that identical parthenogenetic females actually belong to two different categories and it appears reasonable, therefore, to assume the presence of multiple sex categories among parthenogenetic females of open-cycle species. The environment is supposed to act upon such multiple categories of females both by directly influencing the phenotype and by alternatively selecting different genotypes.

Examples of cyclic variations in relative frequencies of different genotypes were given some years ago by Timofeef Ressowsky with his work on *Adalia* (1940) and by Dobzhansky with his extensive research on adaptive changes in natural populations of *Drosophila*. In the case of heterogonic populations, the changing seasonal conditions are supposed to favour certain genotypes until new sex phenotypes (that is mictic females and males) are produced as soon as given thresholds are reached.

The progressive attainment of homozygosis in the succeeding parthenogenetic generations of species, where meiotic parthenogenesis takes place, and the changing adaptive values of the different genotypes with the varying seasonal conditions appear also to have much importance in the delicate interplay between genotypes and environment. The former process leads to the segregation of genotypes which become more and more differentiated as a

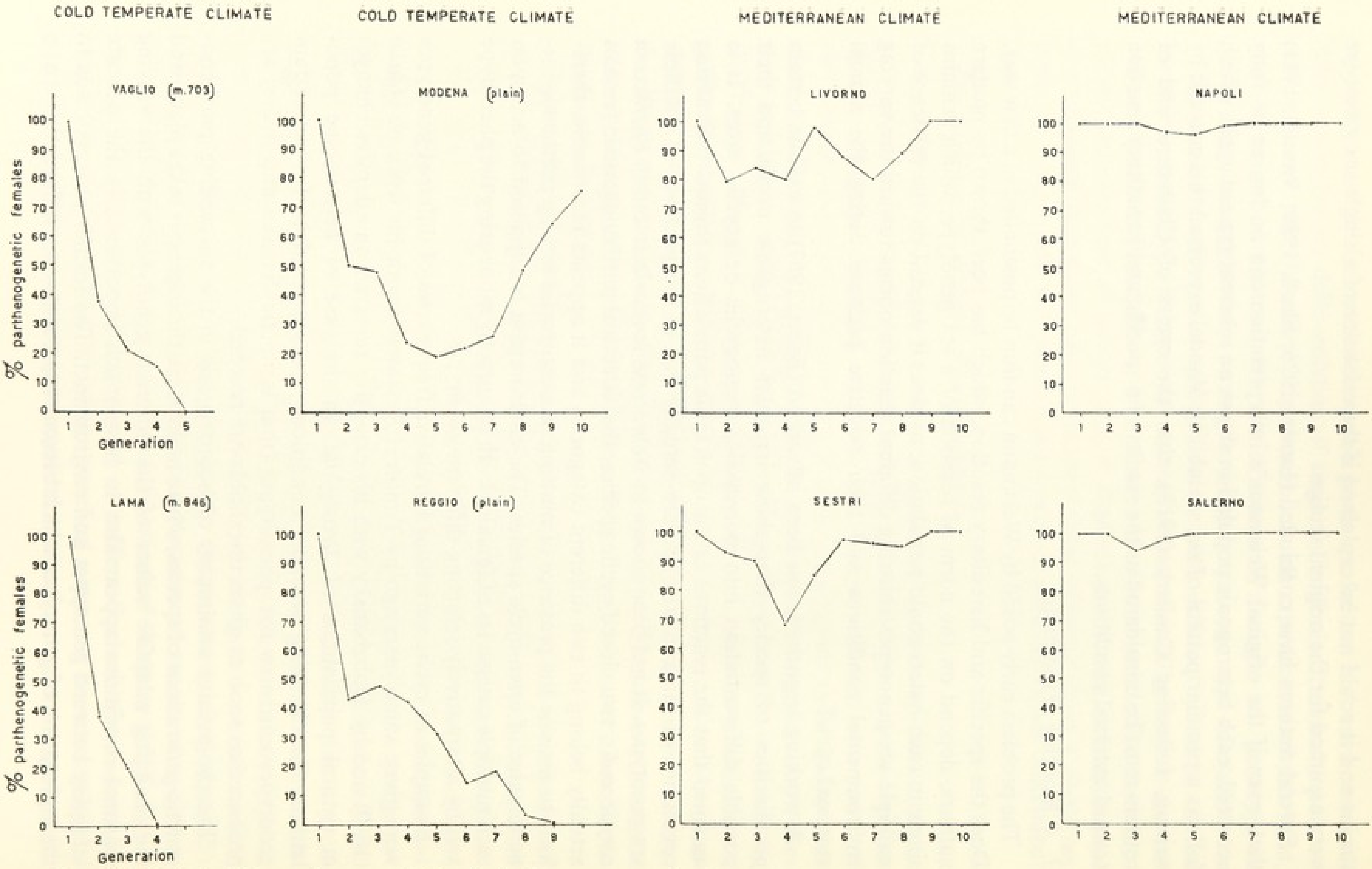


Fig. 11.8 'Selection' for parthenogenetic females in *Brevicoryne* strains from cold temperate and Mediterranean climate (Cognetti, 1962).

consequence of their progressing homozygosis and the latter factor, that is the changes of adaptive values, enhances the effects of such differentiation. The cyclic crises that induce the appearance of amphigonic forms one or more times in the year are thus favoured by the joint action of such factors. Shifts of the balance between the factors favouring parthenogenetic or amphigonic categories give rise, according to the hypothesis, to cyclic or to polycyclic populations, through selection of the different genotypes.

Sex polygamety is thus assumed to be shown by heterogonic populations with irregular cycles as sex digamenty is shown by most closed cycle species. Sex monogamety appears to be shown by acyclic species as the extreme result of a progressive reduction of genetic sex variability.

4. The Brevicoryne *Experiments*

The above hypothesis explains in terms of population genetics the mysterious innate rhythm, which was described by Weismann, and the possibility of its evolutionary change but it had little experimental support until Cognetti and Pagliai investigated the sexual races of *Brevicoryne brassicae* (1964).

Populations of *Brevicoryne brassicae* which live in regions with mild climate reproduce only by parthenogenesis and populations living in cold temperate climates alternate parthenogenetic with amphigonic reproduction.

Individuals which were collected in different localities of the Mediterranean coast of Italy (Mediterranean climate) and in the northern slopes of the Apennines or the Padan plain (cold temperate climate) have been kept in conditions which are apt to induce the maximum production of amphigonic individuals. Selection for parthenogenetic individuals, which was carried on for several generations, led to different results in the strains from cold temperate and from Mediterranean climate (Fig. 11.8).

In three out of four strains from the cold temperate region no parthenogenetic females could be obtained at the fourth, fifth or ninth generation and only from the Modena strain 2 parthenogenetic females survived at the eighth generation. From such females a partially parthenogenetic line could be established. Both amphigonic females and males were produced in the above strains.

All the three strains from the Mediterranean region immediately produced high percentages of parthenogenetic females. No males were ever produced. The strain from the most northern locality (Sestri Levante) produced 84 per cent of parthenogenetic females in nine generations, the strain from the intermediate locality (Leghorn) produced 86 per cent of parthenogenetic individuals and the Naples strain only produced parthenogenetic females.

The results are thus in accordance with the hypothesis and demonstrate different genetic structures of the populations from cold temperate and from Mediterranean climate which have a clear adaptive significance. Statistical analysis will decide whether a gradient can actually be demonstrated between northern and southern Mediterranean populations.

CHAPTER 12

SEX AND EVOLUTION

THE evolution of sex determination in digametic species has been discussed in comprehensive works by Darlington (1932, 1939), by White (1954) and by other authors who have primarily been concerned with the cytological aspects of the sex chromosome mechanisms.

Very little information is available on the other hand about the evolutionary relationships existing among the vast number of organisms which show monogeny, labile gonochorism, relative sexuality, irregular parthenogenetic cycles or hermaphroditism of different kinds. The discussion about evolution from monoecysm or hermaphroditism to dioecysm or gonochorism has scarcely yet begun.

The reason for this situation can be traced to the limited number of observations and experiments which have been made on sex determining mechanisms other than those which are shown by sex digametic species.

It has been a general opinion that organisms which do not show the sex chromosome mechanism represent exceptional cases or at least they belong to a quite distinct category of sex determination (the so-called phenotypic sex determination). For this reason not much interest has been paid to the various aspects of what can broadly be named the polyfactorial determination of sex.

One school of thought discouraged attempts to approach the evolution of sex determination in accordance with the criteria that are used to analyse population genetics. Sex determiners were regarded as quite dissimilar from all other types of genetic factors, or it was assumed that the basic sex determining system is beyond the reach of our present methods of genetic investigation. It is fortunate, therefore, that the presence of detectable sex chromosomes has allowed cytologists to carry out brilliant research within the field of sex digametic species.

Recent comparative research indicated, however, that sex digamety—although it had developed independently in several phyla—is present in a minority of organisms and that mechanisms of sex determination, which were formerly regarded as exceptional, are on the contrary much widespread.

In order to understand why sex inheritance has been regarded by some authors as a "different" form of heredity, it must be recalled that research has concentrated on sex digamety which is obtained through a variety of highly specialized mechanisms. It is not surprising therefore that previous writers have been led to emphasize differences rather than basic similarities between sex inheritance and inheritance of other characters. The recent trend of research points on the other hand to the role of genes, that can hardly be regarded as sex genes, in the simple systems of polyfactorial determination and indicates that it is impossible to draw a straight line of separation across a graduated series of sex determiners which begins with sex modifiers and ends with blocks of sex genes, or supergenes.

Polyfactorial sex determination often implies the possibility of rapid changes in the sexual structure of populations. Such changes are clearly related with a variability of both the sex phenotype and of the sex genotype which, although it is particularly marked in polyfactorial sex determination, is by no means absent in sex digametic populations.

A discussion on the evolution of sex both within and between major systems of sex determination can now be initiated and a few concrete examples be given. Data are rather scarce indeed but a fresh outlook will provide useful indications for future work.

It appears expedient, however, first to discuss a system of classification which takes into account the results on polyfactorial sex determination and on sex in bacteria and can possibly replace the misleading separation into two categories of genetic and phenotypic sex determination.

A Tentative Classification

A classification, even a good one, may prove a useful as well as a dangerous tool because, besides expressing new positive results, it may conceal a lack of knowledge in some important fields. It is important therefore to distinguish between the categories that are mainly based on positive information and the categories that have mainly been established for mechanisms which cannot be made to fit within the boundaries of the other well-investigated categories.

Such is the case of the protosexual as opposed to eusexual and parasexual processes (see Chapter 3). Recombination is brought about in bacteria and viruses through mechanisms that are different from those which have been well investigated in eusexual organisms. It must not be forgotten on the other hand that very little is known about such basic problems as those concerning prophage recombination or replacement of recipient by donor genes after transduction. Until such and other major mechanisms have been clarified, protosexuality will stand as a provisory category whose utility lays only in stressing that cycles of bacteria and viruses are not based on the regular alternation of caryogamy and meiosis and are possibly more primitive than the eusexual cycle. Similar considerations can be made about parasexuality in Fungi as problems posed by somatic crossing-over are far from being solved.

Any discussion on evolutionary relationships between protosexual, eusexual and parasexual cycles would result, therefore, only in vague speculation at the present stage of research.

The widely accepted division of eusexual organisms into the two categories of genotypic and phenotypic sex determination was based on the idea that there is a basic sex determining system which can be put into operation either by the influence of genetic realizators (in the so-called genetic sex determination) or by the influence of external factors (in the so-called phenotypic sex determination). The former category was actually identified with sex digamety and the latter category included all other kinds of sex determination. Kosswig later introduced the notion of polyfactorial sex determination and made possible a discussion of evolution to monofactorial sex determination (or sex digamety). Kosswig's categories represent, therefore, a useful basis for classification as they express a new alternative to the former theory that postulated the existence of either two or a single sex genotype in a population.

A general survey of the sex determining processes in eusexual organisms shows that they result either in the production of multiple sex genotypes, in the production of two sex genotypes or in the production of a single sex genotype. This establishes the categories of sex polygamety or plurigamety, of sex digamety and of sex monogamety. These are based on the degree of sex variability shown by the population and as such they give useful indications concerning sex evolution. Very little is known on the other hand about the actual mechanisms that cause sex polygamety in a number of species and almost nothing about the supposed cases of sex monogamety. The three principal categories are evidently connected by evolutionary steps that are represented by organisms which show intermediate degrees of sexual variability. Each category is represented by different sex conditions which are summarily listed below.

(a) *Sex polygamety*

1. Unbalanced hermaphrodites, illustrated by the researches of Bacci and co-workers (1948–1962).

2. Gonochoric animal species showing monogeny, first illustrated by the researches of Kosswig and co-workers (1933–1959).

3. Haploid organisms showing relative sexuality, illustrated by the researches of Couch (1926).

4. Heterogonic species with irregular cycles, illustrated by Bacci (1957).

5. Labile gonochorists of the *Bonellia* type, illustrated by the newly interpreted researches of Baltzer (1936).

Monoecious plant species like *Ecballium elaterium* or *Cucumis sativus* show marked sex variability and resemble unbalanced hermaphrodite animals in their system of sex determination. The presence of major sex determiners places them in the category of

(b) *Sex digamety*

Sex digamety includes species where the sex chromosome mechanism appears to be decisive in the inheritance and determination of sex. The so-called complementary sex determination, or, determination by haplo-diploidy, appears also to be a highly specialized case of determination through the sex chromosome mechanism.

Some systems of determination in some species of cyprinodonts lie on the boundary between polyfactorial and monofactorial sex determination, that is between sex digamety and sex polygamety.

A still unexplored field, upon which information is still indirect, is represented by the category of

(c) *Sex monogamety*

Sex monogamety includes the following sex conditions:

1. Balanced hermaphrodites of the *Calyptraea* type, which show a single sex phenotype and are supposed to produce accordingly a single sex genotype (Bacci, 1952).

2. Labile gonochorists of the *Ione* type which seem to be devoid of genetic sex variability, according to Reverberi and Pitotti (1952).

3. Acyclic parthenogenetic populations, whose gametes only form parthenogenetic females.

The examples listed in the category of sex monogamety probably do not show genetic sex variability, a situation which will eventually be proved with much difficulty. The descendants of a hermaphrodite population of the self-fertilizing teleost, *Rivulus marmoratus*, seem to comply with such conditions according to researches by Kallman and Harrington (1964) as transplantation tests in thirty-six host donor combinations were successful and indicated that the fish were largely homozygous. Transplants in interline combinations were on the other hand generally not successful. It must be pointed out, moreover, that when a perfect uniformity of the sex genotype has been obtained in some species this can have arisen in two quite different ways. Balanced hermaphroditism and perfect labile gonochorism imply a perfect balance between male and female sex determiners. Stable parthenogenesis appears on the other hand to have been obtained by selection against male determiners in favour of female parthenogenetic phenotypes.

Balanced hermaphrodites, labile gonochorists of the *Ione* type and acyclic parthenogenetic populations appear to be the ending points of different evolutionary series. Some possibilities of reversal to other categories appear open to such categories as total genetic uniformity can hardly be postulated.

The three categories can be considered only as significant steps in a great variety of evolutionary trends of sex determination. They cannot be regarded therefore either as isolated or as definitive categories although further examples will show their utility as reference points.

Sex Variability in Gonochoric Species

Racial sex variability is known in a number of gonochoric species although its analysis has not stimulated much interest in connexion with the problem of sex evolution.

A famous example is provided by *Lymantria dispar* where Goldschmidt was able to establish the existence of races with increasing strengths of sex determiners. Varying valences of male factors were evaluated by mating males from different regions with female individuals obtained from crosses between strong race females (Tokyo) and weak race males (Hokkaido). The crosses showed what can now be regarded as a true sexual cline from the Tokyo to the Berlin population through intermediate degrees of sexual strength in various populations from Japan, Korea and Russia. The Hokkaido race was shown to possess the lowest valence, thus contrasting with the rather high valences prevailing in the more southern Japanese races. When Goldschmidt summarized such remarkable results (1938) he was unfortunately convinced of the cytoplasmic quality of the F determiners and did not accept the theory of race formation which was being elaborated at the time. The results from *Lymantria* remain, however, one of the finest examples of sex evolution within a sex digametic species and the crosses mentioned above add new evidence in favour of the Winge's earlier interpretation.

Research work, which has been mainly carried on with the aim of studying the factors of sex differentiation, has brought about a wealth of information on the distribution of differentiated and undifferentiated races in various species of *Rana*. It is generally assumed—in accordance with Witschi's interpretation (1923, 1929)—that individuals of differentiated races show stronger differences in the balance between male and female determiners than individuals of undifferentiated races. Undifferentiated races seem to represent therefore a primitive condition as compared to differentiated races within each species of *Rana*. Undifferentiated races appear to be widespread in the southern regions within the area of distribution of the species to which they belong but the adaptative significance of such a distribution is not fully clear yet.

Intraspecific sex variability is likely to be particularly high in the species that lie on the borderline between sex digamety and sex polygamety. It has been mentioned before that female digametic populations of *Xyphophorus maculatus* are found in British Honduras and male digametic populations are found in Mexican rivers. Although Gordon demonstrated that sex is determined in *X. maculatus* by major genes, the existence of male and female digamety in different populations that are nevertheless interfertile points to a very low degree of differentiation of the sex chromosomes. A prevailing influence of autosomal sex determiners is shown on the other hand in some strains of unknown origin (Oktay, 1959).

Very little information is available at present on sexual races of unisexual species that show normal sex polygamety.

The observations by Raper (1947) on the mating patterns of *Achlya* strains from Northern Illinois and from England would represent the first information of this kind in haplonts, provided relative sexuality is determined by polyfactorial mechanisms.

Johnson's data on *Haplophtalmus danicus* (1961) demonstrate geographic sex variation in a monogenic species showing polyfactorial sex determination. The most significant results in this field are provided by the selection experiments on *Brevicoryne* (Cognetti, 1964), which have been analysed in the previous chapter (page 237). They show the existence of a sexual cline—which has a clear adaptive significance—from polygametic to monogametic races with a reduction in the frequency of the male genotypes from the northern to the southern populations.

Planned research work, which has just started, will possibly extend such results to other monogenic and parthenogenetic species.

Sex Variability in Hermaphrodite Species

Quite a few data have recently been collected on the variability of sexual differentiation in Atlantic and Mediterranean populations of *Ophryotrocha*.

Figure 12.1 shows the frequencies of the individuals that undergo sex reversal from the male to the female phase at different number of chaetigerous segments (La Greca and Bacci, 1962). The diagram is based on observations on *Ophryotrocha labronica* from Leghorn, on *O. puerilis puerilis* from the Bay of Naples and on *O. puerilis siberti* from Plymouth. Differences in the mean lengths at which sex reversal takes place in *labronica* and in *puerilis* are much marked and are on the contrary only two chaetigerous segments in the Mediterranean and Atlantic sub-species of *puerilis*. It has been suggested (Bacci, 1955a) that the higher body length at which oocytes appear in *puerilis siberti* favours the formation of big ripe eggs which characterize cold water populations. The already mentioned selection experiments showed on the other hand that a shift of two segments can easily be obtained in the system of sex determination prevailing in *O. puerilis*.

The anterior body segments of *Ophryotrocha hartmanni* develop male gonads and the posterior segments female gonads. Parenti demonstrated (1961–1962) that all individuals of *O. hartmanni hartmanni* from Roscoff have only two male segments and that individuals of *O. hartmanni medicea* from Leghorn have either two, three or four male segments. This situation can tentatively be interpreted as an example of sex monogamety and sex polygamety co-existing within a hermaphrodite species.

An even more complex example of sex variation in different populations is provided by *Asterina* which has been studied in Mediterranean and Atlantic waters by a number of authors (Cuénot, 1898; Bacci, 1949; Cognetti, 1954; Neefs, 1956; Delavault, 1960). Sub-specific and specific differentiation of *Asterina* is highly favoured, in the author's opinion, by the absence of pelagic larval stages and by the ensuing genetic isolation of a number of populations. Figure 12.2 shows transverse sections of gonad tubules at different developmental stages in the most significant sex phenotypes which are found in three populations of *Asterina* from the Atlantic (Plymouth) and from the Mediterranean (Fusaro and Baia near Naples). *Asterina pancerii* was formerly regarded as a "variety" of *Asterina gibbosa* but is now considered a separate species: it is certainly a distinct ecotype which seems to be confined to *Posidonia* beds instead of shallow rocky bottoms to which typical populations of *A. gibbosa* are mainly adapted.

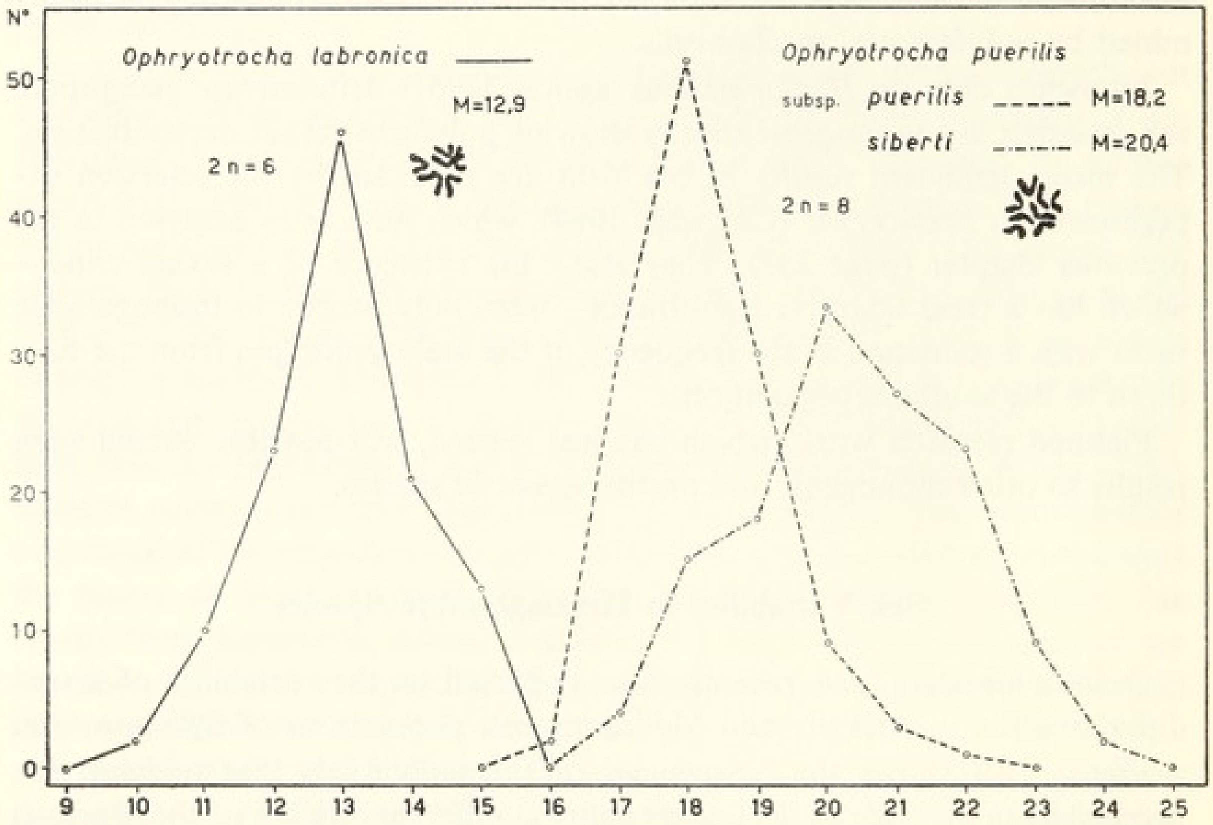


FIG. 12.1. Individuals of the protandrous hermaphroditic *Ophryotrocha puerilis* and *O. labronica* which pass from the male to the female phase at different number of segments (after Bacci and La Greca).

The Plymouth population of *A. gibbosa* shows a phenotype with spermatogonia, spermatocytes and only a few small oocytes in the early stage of differentiation. The gonad tubules are filled with sperms and a few unripe oocytes during the first breeding section. The next breeding seasons bring about the maturation of the oocytes but a few sperms are also generally produced. Only slight variations of this sequence are shown in the Plymouth population with a few individuals which may remain in the male phase during the second breeding seasons or with a few phenotypes which show rather big oocytes, but never ripe oocytes, during the first period of sex maturity (Bacci, 1949). Such conditions were found along the French coast at Roscoff (Cuénot, 1898) and at Dinard (Delavault, 1960) although Neefs showed (1956) a rather high degree of sex variability in a recent study on the Roscoff populations. The variability is certainly much higher in populations from the lake Fusaro near Naples where a minority of individuals remains constantly in the male and a minority in a prevalently female phase (Bacci, 1951). Cognetti showed that such almost pure females resemble in their external phenotype *Asterina pancerii* individuals of the population living in the nearby *Posidonia* beds of

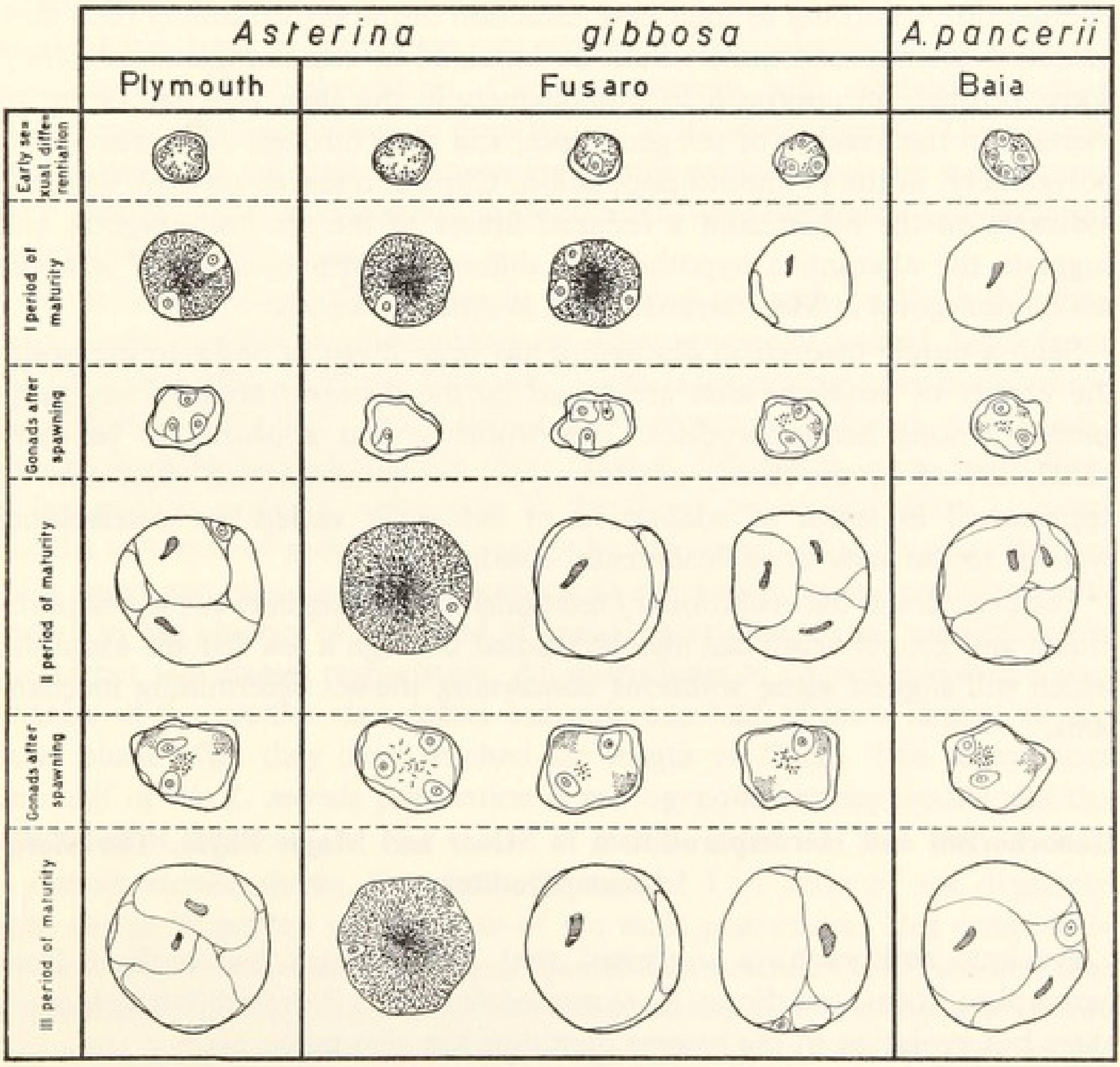


FIG. 12.2. The variability of the sex phenotypes in three populations of *Asterina* (Bacci and Cognetti, 1961).

Baia. The sequence of the sexual phases is also very similar in most *A. pancerii* which shows a marked uniformity of the sex phenotype. The female phase prevails during the whole life cycle and fertilization is ensured in the population by a transient production of sperms which allows self-fertilization after egg laying (Cognetti, 1954).

The Fusaro population of *A. gibbosa* shows the highest degree of sex variability and it can be cited as an example of unbalanced hermaphroditism. Variability is reduced in the Plymouth and in the Dinard population around a protandrous hermaphrodite phenotype and around a protogynous phenotype in the Baia population.

It can be assumed, in accordance with the results on *Ophryotrocha*, that sex is determined in the Fusaro populations through a mechanism of multiple autosomal sex genes. The reduced sex variability may have been obtained through a selection against male factors in the Baia population and through an increase in the number of both kinds of sex factors in the Plymouth population. According to such view reduction of the sex variability (and thus balanced hermaphroditism) would be obtained in two completely different ways: through an approach to monogamety in the Baia ecotype, or by an increase in the number of sex genotypes, and thus through an increased sex polygamety, in the Plymouth population. Current research on *Ophryotrocha* indicates on the other hand a reduced fitness of the sex homozygotes and suggests the alternative hypothesis of different adaptive values of *Asterina* sex homozygotes in Mediterranean and in Atlantic waters.

Such a purely theoretical discussion has been given in order to exemplify the variety of problems that are raised by the demonstration of sex polygamety among hermaphrodites. The problems that appeared to be completely solved by the theory of phenotypic sex determination must now be approached in terms of adaptation of extremely varied sex determining systems to the various environmental conditions.

The problem of the evolutionary relationships existing between hermaphroditism and gonochorism can also be studied through a few specific examples which will suggest some solutions concerning the sex determining mechanisms.

Gonochorism and Hermaphroditism in Minor and Major Phyla. The Merohermaphrodites

Previous authors have suggested that gonochorism has evolved from hermaphroditism, and dioecy from monoecism. This is probably true in most cases but evolution in the reverse direction has also taken place.

The sex conditions of *Anodonta cygnea* from the Rhine basin have been studied by Wiesensee (1916) in a remarkable research which has been carried on at the population level and has been completely overlooked for a number of years. Wiesensee established the following facts:

(a) The running water populations living in the river port of Mannheim showed separate sexes with a 1 : 1 sex ratio.

(b) Populations living in a pond, which had been separate for 27 yr from the Rhine, also showed separate sexes, but with a high proportion of females.

(c) Populations living in ponds, which had been separate from the river for 50 or 60 yr, were hermaphrodite with a small proportion of pure female individuals.

(d) Hermaphrodites only were found in a pond which had been separate 300 yr.

An evolution from the gonochorist to the hermaphrodite condition is evident in this case although there is a possibility that the Mannheim individuals were actually false gonochorists of the *Coris* or *Patella* type. There would have been in such case an evolution from strictly consecutive to simultaneous hermaphroditism. Current research will establish whether false gonochorism is present in populations of *Anodonta cygnea.* The present data provide at any rate a convincing demonstration that sex may evolve very rapidly when hermaphrodite conditions—and probably polyfactorial determination—are concerned.

A recent extension of the already mentioned research on *Coris julis* has led to some significant observations on other species of Labrid fishes from the Leghorn waters. *Labrus turdus* and *L. merula* are protogynous hermaphrodites (Sordi, 1962) like *Coris julis* but the percentages of female phase individuals at different body lengths show marked differences in sex variability. A few pure or primary males exist in the *Coris* population from Leghorn but the existence of pure females cannot be demonstrated at present. Neither pure males nor pure females are present in the *Labrus turdus* populations as shown by the absence of male and of female individuals in the smaller and in the higher size classes respectively. All individuals of *Labrus merula* are also females in the smaller size classes but only one half of them change to the male phase after they have reached the length of 26 cm. This means that one half of the *L. merula* population is protogynous hermaphrodite and the other half is female.

Labrus merula shows, therefore, the typical 1 : 1 ratio of sex digamety with the protogynous in the place of the male phenotype. This example is by no means isolated among animal species as Loosanoff (1937) showed that a population of the bivalve mollusc *Venus mercenaria* is composed of one half male individuals. The male phenotypes undergo a transient intersexual phase during which they carry a few unripe oocytes. A similar sequence has been cited by Danforth (1939) for the pulmonate gastropod *Ariolimax*

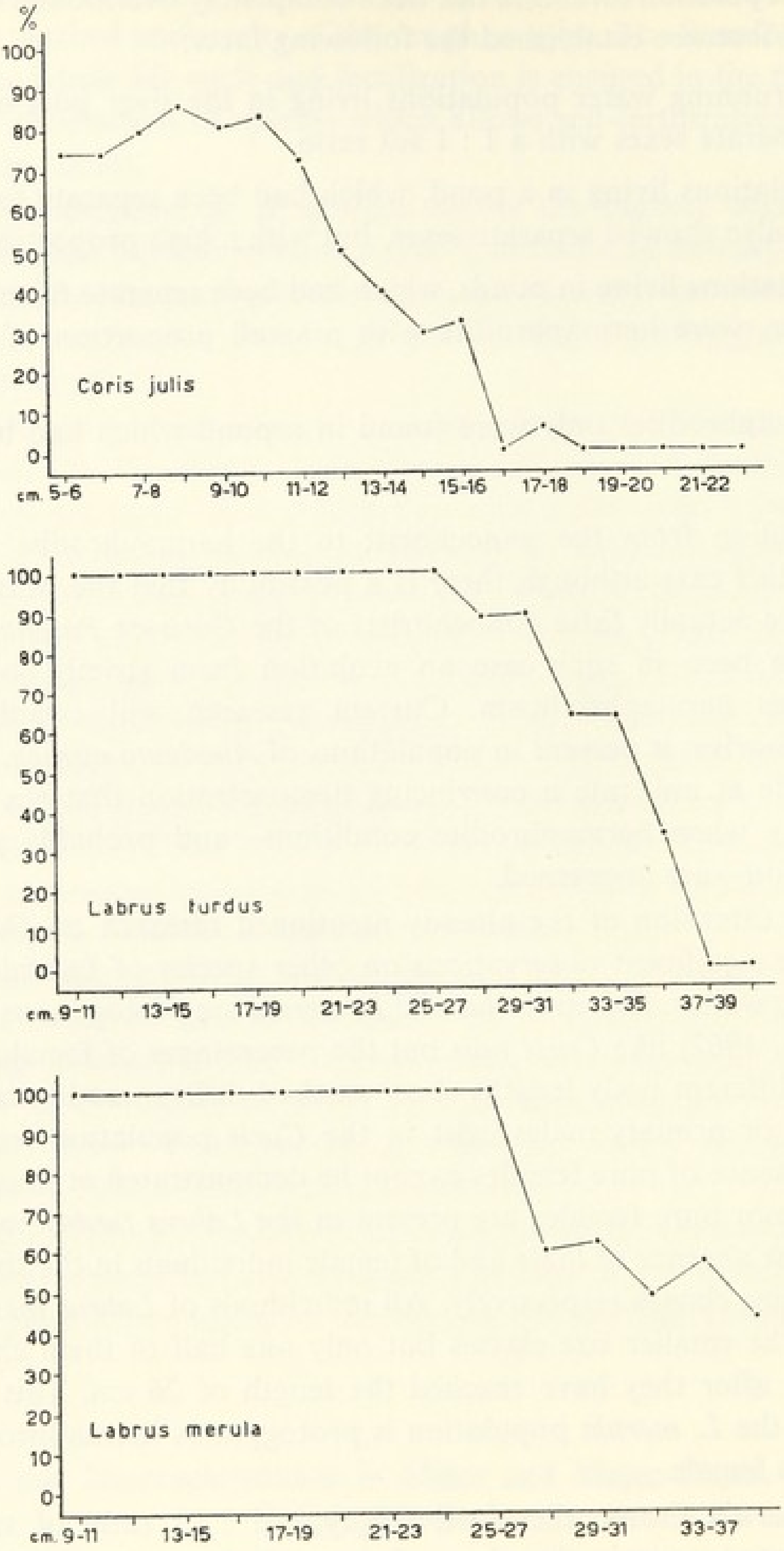


FIG. 12.3. Percentages of female phase individuals in three species of labrid fishes: *Coris julis* shows primary males, all *Labrus turdus* change sex and only one half of *Labrus merula* individuals change to the male phase (after Bacci and Razzauti, 1957 and Sordi, 1962).

californicus. Such species may appropriately be named—in the present author's opinion—merohermaphrodites and they appear to represent an important step in the evolution from hermaphroditism to gonochorism. The hermaphrodite phenotype of the merohermaphrodite species reminds the transient intersexual phenotype in the undifferentiated races of *Rana* and both phenotypes seem to correspond to the male genotype as the female phenotypes do not undergo sex reversal or intersexual phase in their life cycles.

Genetical experiments demonstrated digamety in the male sex of *Rana* and the succession of both sexes in the hermaphrodite phenotype suggest its digamety in the merohermaphrodite species that are known at present. The 1 : 1 ratio of the sex phenotypes points in fact to a sex chromosome mechanism and a close balance between autosomal sex genes and genes located on little differentiated sex chromosomes can be assumed for merohermaphrodite individuals.

More detailed assumptions cannot be advanced concerning the determination of merohermaphroditism. It must be pointed out, however, that labroid fishes belong to the group of teleosts where genetic research has already demonstrated borderline conditions between sex polygamety and sex digamety (see Chapter 6).

A discussion about evolution from hermaphroditism to gonochorism, from monoecism to dioecism, from polygamety to digamety or to monogamety in large phyla cannot be accurate for lack of genetic knowledge and for the scarcity of information upon sex variability.

It has been pointed out (Bacci, 1947) that unbalanced hermaphrodite and pseudogonochorist species are widespread among representatives of the most primitive order of prosobranch gastropods. True gonochorism is shown on the other hand by higher prosobranchs and a highly differentiated type of balanced hermaphroditism prevails among opisthobranch and pulmonate gastropods. Comparative morphological data on gonads in gastropods agree also with the general assumption that both gonochorist and truly hermaphrodite phyla have evolved from phyla where unbalanced hermaphroditism is present. Data on nereimorphs indicate also a large diffusion of unbalanced hermaphrodites in such primitive group of polychaete worms (Bacci, 1950).

A recent survey by Cognetti and Delavault on sex in asteroids (1960, 1962) has demonstrated the widespread occurrence of occasional hermaphroditism in populations of *Echinaster sepositus*, and of other species which are prevalently unisexual. Such labile gonochorist species represent, in their interpretation (Fig. 12.4), a step in the evolution from unbalanced hermaphroditism (of the Fusaro *Asterina* type) to stable gonochorism.

A discussion on the evolution of sex within large phyla can only lead at present to vague conclusions but the work which has been done in this direction points to a high degree of sex variability among the representatives of the lower groups.

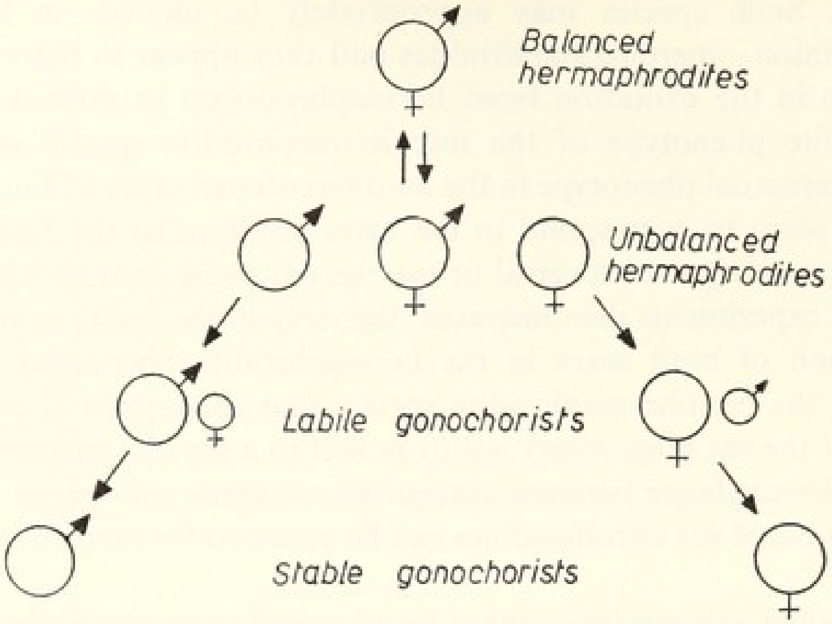


FIG. 12.4. Evolution from unbalanced hermaphrodite condition to balanced hermaphroditism to stable gonochorism in asteroids (Cognetti and Delavault, 1962).

Even more sparse than the facts on the evolution of sex conditions are the facts on the evolution of sex mechanisms.

Some Paths of Sex Evolution

1. Double and Single Systems of Sex Determination

A number of processes, which are more or less closely related to sex determination, have been briefly discussed in Chapter 3. No definite conclusions could be reached, however, concerning the relationships of such systems to the sexual mechanisms which prevail in higher eusexual organisms. The common feature of incompatibility systems (whether they concern the so-called multipolar sexuality or the mating type system) and of the traditional type of sex differentiation lies in that they all help in bringing about amphimixis, that is they increase the chance of crossing between individuals with different genotypes. If bipolar sex differentiation, the mating types, the so-called tetrapolar or multipolar sexuality are equally to be regarded as sexual systems on the basis of such evolutionary principle, then the definition of sex by the traditional morphological and physiological criteria loses its formerly pre-eminent importance.

The author is not unwilling to follow this view which avoids the difficulty of accepting the concept of the purely theoretical "allegemeine bipoläre Sexualität" (Hartmann, 1931) and the unnecessary notion of the AG system (see Chapter 4). It must be pointed out on the other hand that in such case the somewhat startling notion of double systems of sex determination must be accepted. Such would be the case of mating types and sex differentiation in the "hermaphrodite" Infusoria and the case of parasexual and eusexual processes in some Fungi. The complete diversity of sexual differentiation in protosexual and eusexual organisms suggests the Weismann–Müller theory as the basic one for sexual systems.

The extremely diverse systems which are at work among lower eusexual organisms in carrying on amphimixis include in most phyla some form of differentiation into male and female sex. The data available indicate that sex differentiation (which is traditionally identified with sex) asserted itself in many ways and has ultimately supplanted all other systems among higher organisms where a single system of sex determination prevails. Hartmann and co-workers did much valuable work in pointing to the existence of the traditional bipolar sex differences among lower algae and Blakeslee carried out similar studies on Fungi but recent research points to the danger of regarding any difference among gametes, gametangia, hyphae or gamonts as the expression of sex differences in the way which is established among spermatophytis and among Metazoa. Maleness and femaleness appear to have arisen by degrees in different phyla and it is reasonable to assume that the same has happened for the sex determining factors. Our limited knowledge of sex systems in lower organisms does not allow any more definite conclusions.

If the above views are correct—the data available are very scarce—maleness and femaleness are not to be regarded as essential features of the organisms but are the results of evolutionary trends which have been more or less successful in different phyla.

It is certain at any rate that a unified theory of sex determination is impossible at present. The simple schemes that have been proposed so far cannot be unified. It appears that this situation is due to the existence of different and sometimes diverging, evolutionary trends in the determination of sex.

2. *Origins of the Sexual Differentiation and the Sex Ratio Factors*

Kalmus and Smith (1960) have taken the amphimixis theory as a starting point for their interpretation of the evolutionary origin of sexual differences. They include sex differentiation *sensu strictu*, self-incompatibility of Fungi and mating types of Infusoria under the common denomination of "sex" and say that the function of sex is that of reducing inbreeding so far as possible. All the above expressions of sexuality would ensure a more or less precise equality of two kinds of gametes and also confer a selective advantage by allowing a maximum gametic diversity of the population. The evolution of sex differentiation is thus closely linked in this interpretation with the attainment of the advantages of numerical sex equality.

The authors examined some simple cases of sex determination, which actually conceal delicate mechanisms of genic balance, and suggested theoretical possibilities for obtaining two sexes with equal frequency. One possibility is the appearance of a dominant gene *A* for large gametes in a diploid population homozygous for gene *a* which produces smaller gametes. The spread of the gene *A* would be checked by the difficulty of fertilization between relatively static gametes, whose advantage (which consists in producing large zygotes) remains only if a sufficient number of smaller *a*-carrying gametes is produced by the population. This situation would lead to a numeric equilibrium between large and small gametes and would ultimately result in a true sex differentiation. Also a dissortative mating with a preference for the dominant x-recessive types would lead to similar results.

The Kalmus–Smith hypothesis provides a simple interpretation for the origin of sex polarity without having recourse to the myth of the primordial male and female principles. The interpretation is based on the other hand on the current theories of "monofactorial" sex determination although relative sexuality—which is apparently determined by multiple sex genes—most closely approaches the conditions which have been suggested by the authors. It appears, therefore, that the development of the theory on the basis of the results of polyfactorial sex determination would lead to a closer approach to the real conditions of early evolution of sex differentiation.

The selective significance of the sex ratio genes has also been discussed with relation to results on multifactorial sex determination in isopods (Shaw, 1958). The already cited results on *Ophryotrocha* showed on the other hand that autosomal genes influencing sexual differentiation produce monogenic female or male strains when in homozygous condition and it appears that hereditary patterns of sex differentiation decide whether sex genes will function as sex differentiators or as sex ratio determiners.

Sex ratio factors may belong to quite different types like the X-linked gene of *Drosophila pseudoobscura* or the infective factor which has been investigated in several *Drosophila* species. The study of various possibilities that may be presented by autosomal and X- or Y-linked sex ratio genes presents new evidence for the assumption that the problems of sex ratio and sex differentiation are susceptible of multiple solutions and have been solved in multiple ways in the course of evolution.

3. The Diversity of Genetic Sex Factors, Sex Chromosomes and Heterochromatin

Attempts to build up a genetic theory of sex determination which could be unified and simple in spite of all variants, have failed, in the present author's opinion, for two main reasons.

It has been mentioned already that previous theories were essentially based on the knowledge of the very restricted field of sex digametic organisms. This regarded sex factors as highly specialized entities and a number of other factors like modifiers, multiple sex genes, realizators, trigger genes, etc., were often regarded as being essentially dissimilar from sex genes proper and only accidentally related with them in the course of sex evolution.

No satisfactory definition of the sex gene is available from the current literature nor can one be provided unless genetic sex factors are regarded as dynamic and not static entities. It appears that the various kinds of sex influencing factors belong to a complex evolutionary series which has reached the lowest levels in the simplest sex determining systems and is organized in a series of hierarchical fields which may even include a chromosome as a whole, in the highly specialized systems of the sex digametic organisms. Recent genetic research has led to the definition of units such as the cistron, the recon and the muton and it appears now that sex factors will represent excellent material to investigate the spatial and structural relations of larger parts of the chromosome, as shown by the Pipkin's and Westergaard's experiments in *Drosophila* and *Melandrium* respectively. Blocks of sex genes or supergenes cannot always be regarded as merely mechanical units but rather as chromosome sections where a repatterning of the original structure has taken place especially when heterochromatin is present. For this reason sex determiners of *Drosophila melanogaster* seem to behave as multiple sex genes according to some experiments (Dobzhansky and Schultz, 1934) or as indivisible units according to other experiments (Pipkin, 1942–1960). Even in less specialized systems, like those of *Lebistes* or of *Xiphophorus maculatus*, it has been impossible to decide whether sex determiners are formed by single or by closely linked loci.

Once the idea that sex determiners can reach different levels and follow different trends in the course of evolution has been accepted, the distinction between a trigger mechanism and a basic sex determining mechanism (as was shown in *Melandrium* by Westergaard) can easily be understood from an evolutionary point of view. The factors that inhibit the action of one and enhance the action of another set of sex determiners or sex promoting genes of *Melandrium* only apparently resemble the sex realizators of the Wettstein–Hartmann theory and, on the other hand, there is no need to stretch Westergaard's results in order to fit them onto a peculiar sex balance theory.

The majority of available experiments shows that sex chromosomes, as all sex factors, may work differently in different species and may have undergone different evolution. It has not been generally realized that sex chromosomes act as sex determiners in some species like *Sciara coprophila*, *Phylloxera caryaecaulis* or the aphids but they have no influence on the sex ratio, which is controlled in *Sciara* and *Phylloxera caryaecaulis* by unknown factors and is controlled among the aphids by an interaction between multiple sex genes and environment. Gonochorism and sex digamety appear to have arisen in different ways even when sex chromosomes are both sex differentiating and sex ratio determining: the cases of *Rumex acetosa* with its *Drosophila*-like type of sex determination and of *Melandrium album* with its peculiar type of determination are illuminating in this respect especially when considering that they belong to the predominantly monoecious group of angiosperms and have consequently undergone a recent evolution to monoecism.

The trigger that ultimately directs the process of development in one or the other of the alternative paths of sex is controlled by a simple quantitative ratio according to the balance theory. Such balance is not obtained, however, either through a similar way of action of the sex chromosomes in all species or necessarily through the presence of sex chromosomes.

It was early recognized that sex chromosomes are often rich in heterochromatin (Heitz, 1928) especially in those segments which are concerned with sex determining processes. Goldschmidt (1956) remarked that in *Drosophila melanogaster* almost all intercalary heterochromatin is concentrated in the left section of the X chromosomes. This is in accordance with the elusive character of sex factors that determine continuous quantitative variations rather than sharply defined and clear-cut qualitative characters. Goldschmidt found that heterochromatin could not be demonstrated in the Y chromosomes of *Lymantria* nor of *Bombyx mori* although they have a strong female determining influence (Tanaka, 1939). This appears to be another indication of the different character that sex factors can assume in different organisms.

There are many indications indeed that heterochromatin is involved with the activity of sex genes but it must be acknowledged that the present crude notion of heterochromatin probably groups a number of different chemical and structural functions within the sex chromosomes. The suppression of the crossing-over in the sex chromosomes, the presence of heterochromatin, its possible influence in integrating sex genes and sex modifiers into higher sex determining units seem to represent one of the most fascinating problems for future work in genetics. The recent discoveries on the inactivated X chromosomes in mammals (see Chapter 5, page 111), to which the lecanoid type of heteropycnosis (see Chapter 10, page 209) can probably be compared—represent in the author's opinion a very promising way of approach to the problem of higher sex determining units and to the problem of the activity of units higher than the cistron.

The problem of how the structural hybridity of the digametic sex can be obtained through evolution from polyfactorial systems of sex determination appears of minor importance.

4. Sex Homozygosy and Heterozygosy: Haldane's Rule

Real understanding of sex evolution started when it was realized that sex chromosomes developed through restriction of the crossing-over (Darlington, 1931) which brought about the structural hybridity of one of the sexes. The study of sex evolution among sex digametic species developed much along such lines with the discovery that crossing-over is often reduced in all the chromosomes of the digametic sex and with the consequent interpretation of the origin of male haploidy in coccids (Hughes Schrader, 1948). The origin of the multiple sex chromosomes, the evolution from the XY to the XO mechanisms could also be interpreted on the basis of Winge's discovery (1923) and have briefly been discussed in previous chapters.

The first genetical interpretation of the evolution from mono to dioecy was provided by the work on silkless and tassel seed genes in *Zea mays* (Jones, 1934) and it has been shown that such mechanism appears rather improbable for the number of conditions that should simultaneously interact in such instance.

Kosswig (1933) made the first important step in understanding the evolution from autosomal to sex chromosome determination by demonstrating the homozygosy of the male and of the female sex of *Xiphophorus helleri* and indicated that from such condition either male or female digamety can originate. That such is really the case has been demonstrated by a number of later works. The discovery of the influence of colour genes upon the sex ratio (Kosswig, 1933a) led to discussions about sex genes and modifiers. It has already been suggested that such apparent problems can be solved by assuming a multiple origin of sex factors, and the possible integration of sex modifiers in the system of proper sex determining factors. It has been mentioned in previous chapters that the work of Kosswig and collaborators has been criticized mainly on the ground that a polyfactorial system of sex determination is not stable enough for so constant a thing as sex. Such criticism was advanced at a time when homeostatic mechanisms were little known in polygenic systems. A recent note by Battaglia (1963) suggests for instance the existence of selective mating between heterozygous females and homozygous males in *Tisbe reticulata.* This observation fits with the already mentioned hypothesis by Kalmus and Smith and it suggests a factor of evolution from the homozygous condition of both sexes in polygenic sex determination to the heterozygosy of one sex in sex digametic populations.

The study of unbalanced hermaphrodites and their polyfactorial type of sex determination (see Chapter 8) (Montalenti, 1950; Montalenti and Bacci, 1951) suggested the hypothesis of a couple of genes assuming the role of major genes with the remaining genes assuming the role of minor genes. One of the homozygotes would be a male or a female according to the type of digamety, the other would be lethal or sterile and the heterozygote would be a female or a male individual, with the remaining genes eventually influencing toward the one or the other sex. The hypothesis strongly recalls, in the author's opinion, the situation found in *Xiphophorus maculatus* with minor factors inducing sex reversal in some strains (see Chapter 6). Such examples suggest that suppression of crossing-over in a chromosome section carrying a prevalent quantity of genes for one sex may have led to a gene block simulating a major sex allele. And male or female digamety may have been developed according to the prevalent sexuality of the regions where crossing-over has been suppressed. The present alternative hypothesis appears to agree with the presence of male and female digametic populations in *X. maculatus* and with the general observations that both male and female digamety are observed in groups where sex polygamety is also present. Similar types of evolution have probably developed sex digamety among many phyla of Metazoa. Oligogenic sex determination may also represent an evolutionary step between poly and monogenic determination.

The dioecious breeding systems have probably had quite different origins among angiosperms from plants that were originally dioecious (Darlington, 1956.) The system of recessive genes and dominant suppressor blocks which has been studied by Westergaard in *Melandrium* represent one of the ways to attain dioecism. Another system is represented by the spinach plant in which, according to Janick and Stevenson (1955), the major factors determining sex expression would be represented by alleles at a single locus. It appears that the few dioecious angiosperms have arisen independently in different phyla.

The recent work on *Brevycoryne brassicae* (Cognetti, 1963) has confirmed the hypothesis that acyclic parthenogenesis can be developed from originally sex plurigametic populations through natural selection against the male genotypes (Bacci, 1957).

The possible ways of origin of balanced hermaphrodite populations (Montalenti, 1950; Montalenti and Bacci, 1951) have been discussed in a previous chapter and they require much further work in order to be satisfactorily tested.

After discussing the evolution of sexual systems it is worth mentioning some of the data that have been accumulated about the sex of species hybrids. The most significant facts have been expressed by what is currently known as Haldane's rule (1922) which says that the hybrid individuals of the digametic sex are in general less viable and less fertile than those of the homogametic sex. The data on which Haldane based his conclusion were largely drawn from Lepidoptera and bird hybrids which showed an excess of females. The X-linked genes interact with autosomal genes and produce lethal or sterility effects in the hybrids when the corresponding dominant alleles are absent, as was early suggested by Haldane, who excluded the influence of the sex factors.

The type of sex digamety of two species can consequently be deduced from the sex percentages of their hybrids as in *Triturus* whose interspecific crosses produce mostly female offspring (Benazzi, 1946–1960; Spurway, 1953) thus suggesting a male digamety of the genus *Triton* in contrast with the female digamety of other genera of urodeles like *Amblystoma* and *Pleurodeles* (see Chapter 4). The prevalence of the male sex has on the other hand been observed also in interspecific hybrids of *Rana* (Kawamura, 1949) where male digamety has been firmly established. Other exceptions to Haldane's rule have been found in different organisms and various explanations have been proposed for the separate cases. A new interpretation is suggested now for the *Rana* case which may prove interesting for other genera with little differentiated sex chromosomes. If the Haldane effect is due to the interaction between genes on the X chromosome and those in the autosomes it can be expected that the number of such genes will be scarce when the differential segment of the X chromosome is short, in other words when the sex chromosomes are little differentiated. The sex ratio of the hybrids will be influenced in such case by factors other than sex digamety, as in the case of *Rana* hybrids. If such explanation is right the Haldane effect can be used to evaluate the degree of differentiation of the sex chromosomes. That sex ratio can be influenced in hybrids by factors other than sex digamety can be deduced from a number of other indications, among them the fact that among hybrids of hermaphrodite plants male sterility is more frequent than female sterility (Soost, 1951) the male sex organs being in such cases more sensitive than female organs to genetic influences.

5. *Genetic Sex Factors and the Environment*

The various genetic mechanisms of evolution which have been discussed in this chapter would not work if they had not some selective advantage in the environment where the sexual populations reproduce. The dynamics of sex evolution cannot be understood therefore unless the selective advantages of certain sexual systems are investigated.

The advantage of the 1 : 1 sex ratio has early been the subject of close consideration (Fisher, 1931) and a number of papers has been published on the theoretical genetics of the sex ratio. Such work is also useful for the planning of research on polygenic sex determination.

The adaptative significance of different sex conditions has, on the other hand, been the object of some speculations but not of much serious work. The advantages of hermaphroditism and of labile sex determination in parasitic organisms have been pointed out by a number of authors. Labile sexuality is evidently important for the sessile female *Bonellia* in order to ensure fertilization from males that can develop from undifferentiated larvae. A certain number of genetically determined male and female larvae ensures successful colonization of isolated territories. Slowly moving animals are also said to favour hermaphroditism, as shown by flatworms, earthworms, leeches and pulmonate land snails.

Parthenogenetic reproduction presents clear advantages for a quick exploitation of food resources at the beginning of the breeding season. The studies on *Brevycoryne* showed that the production of males and of mictic females is genetically determined in relation to the seasonal cycles prevailing in different regions. Weismann's interpretation has now become ripe for further study.

The study of the genetical basis of the apparently "non-genetical" mechanisms that are still being commonly included under the name of phenotypic sex determination must now be extended and correlated with comparative research on sex factors from modifiers to the sex chromosomes.

External agencies do modify the sex phenotype but their basic influence is exerted through selection on the sexual structure of the populations. They may even induce the genetic assimilation (Waddington, 1956) of some new sex characters. Integration between nature and nurture would thus result in sex determination.

Unitary theories lead to consideration of either a double system of sex determination or a basic difference of the sex factors from all other genetic factors. Comparative research shows a multiplicity of sex determiners and of sex determining systems which are different from all other genetic factors and mechanisms only insofar as any biological process is different from all others. The apparently simple character of sex determination results from the very multiplicity of its systems and these systems lead to a dynamic balance that can adjust with environmental conditions the more or less variable.

APPENDIX

The present trends of research on sex determination

SOME important work has been published in the interval between the consignment of the manuscript of this book and the correction of the first proofs. An attempt to show the most promising fields of present day research is made in the following pages.

Kosswig (1964) has published a comprehensive account of results and problems of polygenic sex determination. The paper is primarily concerned with the work on Cyprinodont fishes and on Isopods and it contains quite a few considerations that appear important for future research. Kosswig rightly points out that sex genes with different quantitative effects may be involved in a polymeric system of sex determination and that sex loci may have more than two sex alleles. Examples supporting such a view can indeed be found in previous chapters and they show the danger of oversimplifying the problems of polyfactorial sex determination.

Kosswig reports additional evidence for the existence of strong and weak males which is based on work by Peters on *Xiphophorus helleri* strains from Honduras. Crosses of early differentiating and of late differentiating males with the same female have given different results, that is more males are produced in the progeny of the early, than in the progeny of the late, males.

Such phenotypic differences among males with different sex genotypes represent thus a connecting link between the strong males of the unisexual and the so called pure males of the unbalanced hermaphrodite populations.

The problem of homeostasis in unisexual populations showing sex plurigamety is still open, as Kosswig points out, but the recent results on *Ophryotrocha* (see Chapter 8) suggest looking for a solution in the study of gonadal differentiation.

New models of sex chromosome structure such as those investigated by Beermann (1955) in *Chironomus*, by Tinturier Hamelin (1963) in *Idothea* and by Yamamoto (1964) in *Oryzias* now suggest various ways of evolution from the sex determining mechanisms that prevail in the plurigametic to those that prevail in the sex digametic populations. A sex chromosome like structure which has been described in the rhyzocephalian species *Peltogasterella socialis* by Yanagimachi (1961) is reported to be exclusive of the small female producing eggs. Such observations recall similar findings in the life cycle of Phylloxerans but the appurtenance of *Peltogasterella* to a group where hermaphroditism is prevalent makes the interpretation rather difficult at present.

The integration of cytological with genetical research is still strongly furthering our basic knowledge on sex determination. The somewhat novel concept of isochromosome—which is composed length-wise of identical halves—is assuming more and more importance in the ever-growing literature on sex anomalies in man. Human caryotypes have been described which are made up by a normal autosome set, by a normal X and by a long metacentric chromosome, which has the same parameters as the 3rd pair of the Denver system. This last chromosome has been interpreted as an isochromosome and is being indicated with a large X_1. The discovery of sex mosaics containing such chromosomes in some tissues and not in others (Lindsten 1961) complicates the picture from the point of view of the action of the X chromosome segments.

Substantial advances in the study of sex determination in man can now be expected from the study of replication patterns of human chromosomes (German 1962, Schmid 1963), which is based on the method introduced by Taylor and co-workers in their research of DNA synthesis in *Vicia faba* (1957). As thymidine is one of the necessary components of DNA synthesis, radioactive thymidine is offered the cells and the time at which the substance is taken up can be determined with some accuracy. It has thus been possible to demonstrate that DNA replication is going on in the allocyclic X of the female tissues when replication has already ceased in autosomes and in the other X chromosome. The allocyclic and the homocyclic X are thus often referred to as the "hot" and the "cold" X respectively.

Research concerning replication of heterochromatin in interphase nuclei of man (Lima de Faria *et al.* 1965) shows interesting relationships between nucleolus associated, scattered (or autosomal) and sex chromatin.

Comparative work in placental mammals (Ohno and Beçak 1964) indicates that, in spite of wide variations in chromosome number, about the same amount of genetic material is found in a significant sample of mammalian species. The X chromosome of most species is almost identical in absolute size, but on the other hand it is twice the basic size in the golden hamster (*Mesocricetus auratus*) and three times that size in the creeping vole (*Microtus oregoni*). Ohno distinguishes therefore three X chromosome types among Mammals. They demonstrate distinctive behaviour patterns in somatic cells with the males of the basic type X chromosome having the X chromosome entirely euchromatic and the females with only one condensed X chromosome. The males of the duplicate type X chromosome (*Mesocricetus*) have one half X condensed and the females one and a half condensed X. Both male and female individuals of the triplicate type (*Microtus oregoni*) have only one X in their somatic cells and about two thirds of the X is positively heteropycnotic.

Ohno reaches the conclusion that all placental mammals seem to contain the same amount of X euchromatic material and that this result is obtained through the inactivation of different lengths of the X in the three types of X chromosomes. A fixed X/A ratio would thus be presented both by male and female genotypes of all placental mammals, at least as far as euchromatic segments are concerned. This appears indeed a marked deviation from the situation found among Insects and it renders more and more desirable an extensive verification of the Lyon's hypothesis in a number of mammals.

Commoner (1964) has translated in terms of molecular genetics the prevailing ideas on the euchromatic and heterochromatic chromosomal sections and assumes that DNA participates in two systems of genetic control. The first is localized in the euchromatic regions and it is concerned with mendelian inheritance, the second is located in the heterochromatic regions and it determines mainly quantitative differences through its control of oxidative metabolism and of other metabolic processes. Mittwoch (1964) points out therefore that the sex chromosome mechanism of Mammals represents a quasi-schematic example of the dual system of genetic control postulated by Commoner's work.

Narbel Hofstetter (1964) reviews the most significant work on the alterations of meiotic processes in parthenogenetic animals. Much research illustrated in the monograph confirms the view expressed in Chapter 11 that it is difficult to separate meiotic (or antomictic) from ameiotic (or apomictic) parthenogenesis on purely morphological grounds and that classification of biological processes are often very provisory. Thus endomeiosis of Aphids (see Chapter 11) was classified as an example of ameiotic process regardless of the fact that Cognetti showed that the variability of the parthenogenetic lines of Aphids is a consequence of meiotic recombination. A more extended practice of integrating genetical investigation with detailed morphological research will eventually overcome difficulties which have nevertheless a prevalently semantic character.

The analysis of Charniaux Cotton (1964) on factors controlling sexual differentiation of higher Crustaceans demonstrates more and more clearly the sex deciding function of the androgenic glands through its control upon the different genes that, in Charniaux Cotton's interpretation, control male characters, ovarian differentiation, female external characters and are either favoured of inhibited by the presence or absence of the androgenic gland which is controlled in turn by the proper sex determining genes. This interpretation implies therefore, as already suggested in Chapter 9, an inhibitory action by the genes determining the female sex upon the development of the androgenic gland in the female genotype and implies also the existence of distinct genes for ovarian differentiation and genes for female external characters both in the female and in the male genotypes. Charniaux Cotton regards as unnecessary for explaining sex determination in Crustaceans, the notion of genic balance, although sex bipotency has been demonstrated again by the work of her collaborator Berreur Bonnenfant (1964) who has obtained ovarian development from testicular tissues of *Talitrus* which were cultivated in vitro in the absence of the androgenic gland. The use of embryological techniques for the study of Crustacean sex determination appears thus more and more illuminating from the point of view of the action of the sex genes although the assumption of a series of different genes for sexual differentiation requires further verification.

A general reappraisal of both genetical and physiological data has allowed Köhler (1964) to extend Heslop Harrison's (1961) hypothesis into a general theory of sex determination in flowering plants. The already mentioned Goldschmidt's idea of male and female reaction's curve (see Chapter 9) and the effect of auxin on the sexuality of various plants are at the basis of the theory. It is assumed that there is a single A substance which is sex realizing and that its function can be assumed by auxin. The reaction chains for anther and pistil production, that is the F and M reaction curves, show distinct optimal values with regard to such A substance. On the other hand both genetic and environmental factors influence the concentration of the substance in the floral meristems and thus they influence sex. After a short survey of the genetics of sex determination in *Melandrium, Rumex, Cannabis, Bryonia, Ecballium*, etc. Köhler has shown the formal applicability of the theory to the existing experimental data.

It appears to the present author that the A substance has more the character of an evocator, as animal embryologists regard it, than that of a sex realizing substance. Substances which are formed in the supposed anther and pistil reaction chains should on the other hand be regarded as sex realizing. The whole question remains, however, at a very hypothetical level although Padoa's verification of the F and M reaction curve in amphibians renders it very plausible and a good starting point for future work on plants. Research reviewed and partially carried on by Köhler himself has, on the other hand, ascertained the important fact that different concentrations of such normal products of the plant organism, as the auxins, and other substances, have a decisive influence on the sex determining systems.

This enhances our interest in the results concerning aspecific environmental influences both in plant and in animal sex determination (Triantaphyllou and Hirschmann 1964). The demonstration of highly specific environmental factors in sex determination (Parenti 1965) shows on the other hand that environmental influences on sex parallel the various types of genetic sex influencing factors. The possibility for a genetic assimilation of external sex determining influences appears therefore more and more consistent.

As a conclusion on present ideas about sex it may be mentioned that Hayes (1964) does not regard sexuality of bacteria as having a high evolutionary significance. Hayes remarks that sex is a rare property among bacteria where it is of a rather rudimentary type. It can be regarded therefore as an insignificant source of variation in populations which multiply with extreme rapidity so that the variability obtained through mutations can successfully cope with changing environmental conditions. The opinion of a leading investigator in bacterial genetics adds therefore new weight to criticism against overemphasis on the importance of the amphimixis theory.

The study of sex of bacteria and viruses has led, however, to the present advances in our knowledge of the genetic material at the molecular level. Future investigation on the structure and chemistry of the varied sex determining factors will certainly take advantage of such results.

REFERENCES

Abeloos, M. (1950) Changement de sexe et comportment sexuel de *Platynereis dumerili*, *C. R. Acad. Sci.* **231**, 179.

Aida, T. (1921) On the inheritance of color in a freshwater fish *Aplocheilus latipes*, with special reference to sex-linked inheritance, *Genetics* **6**, 554–573.

Aida, T. (1930) Further genetical studies of *Aplocheilus latipes*, *Genetics* **15**, 1–16.

Aida, T. (1936) Sex reversal in *Aplocheilus latipes* and a new explanation of sex differentiation, *Genetics* **27**, 136–153.

Allen, B. M. (1907) An important period in the history of the sex cells of *Rana pipiens*, *Anat. Anz.* **31**, 339–347.

Allen, C. E. (1917) A chromosome difference correlated with sex differences in *Sphaerocarpus*, *Science* **46**.

Allen, C. E. (1935) The occurrence of polyploidy in *Sphaerocarpus*, *Amer. J. Bot.* **22**, 635–644.

Anders, F. (1957) Über die Geschlechtsbeeinflussende Wirkung von Farballelen bei *Gammarus pulex* ssp. *subterraneus*, *Z. Vererbungslehre* **88**, 291–332.

Anderson, T. F. (1958) Recombination and segregation in *Eschericia coli*, *Cold Spring Harb. Symp.* **23**, 47.

Anderson-Kotto, I. (1936) The inheritance of apospory in *Scolopendrium vulgare*, *J. Genet.* **32**, 189–228.

Arcangeli, A. (1931) Sul rapporto numerico dei sessi negli Isopodi terrestri. *Boll. Mem. Zool. Anat. Comp. Univ. Torino* **41** (3).

Arcangeli, A. (1932) L'ermafroditismo negli Isopodi terrestri, *Arch. Zool. Ital.* **17**, 165–256.

Artom, C. (1931) L'origine e l'evoluzione della partenogenesi attraverso i differenti biotipi di una specie collettiva (*Artemia salina* L.), *Mem. Acc. Italia* **2**.

Bacci, G. (1947a) Ricerche preliminari sul sesso di *Patella coerulea*, *Arch. Zool. Ital.* **31**, 293–309.

Bacci, G. (1947b) L'inversione del sesso e il ciclo stagionale della gonade in *Patella coerulea*, *Pubbl. Staz. Zool. Napoli* **21**, 183–214.

Bacci, G. (1947c) Osservazioni sulla sessualità degli Archaeogasteropoda, *Arch. Zool. Ital.* **32**, 329–341.

Bacci, G. (1949a) Ricerche su *Asterina gibbosa*. II. L'ermafroditismo in una popolazione di Plymouth, *Arch. Zool. Ital.* **34**, 49–74.

Bacci, G. (1949b) Osservazioni statistiche sulla determinazione dei sessi in *Patella coerulea*, *Pubbl. Staz. Zool. Napoli* **22**, 26–39.

Bacci, G. (1950a) Alcuni problemi dell'ermafroditismo negli Invertebrati, *Boll. Zool.* **17** (Suppl.), 193–212.

Bacci, G. (1950b) Osservazioni sulla sessualità dei Nereimorfi, *Boll. Zool.* **17**, 55–61.

Bacci, G. (1950c) Culture di microrganismi autotrofi e di piccoli animali marini, *Boll. Zool.* **17**, 49–54.

Bacci, G. (1951a) Ermafroditismo e intersessualità nei Gasteropodi e Lamellibranchi, *Arch. Zool. Ital.* (Suppl.) **7**, 57–151.

Bacci, G. (1951b) L'ermafroditismo di *Calyptraea chinensis* e di altre Calyptraeidae, *Pubbl. Staz. Zool. Napoli* **23**, 66–90.

Bacci, G. (1951c) Effetti di ripetute amputazioni sulle fasi sessuali di *Ophryotrocha puerilis*, *Boll. Zool.* **18**, 193–196.

Bacci, G. (1951d) Existence of true males and females in a hermaphrodite population of *Ophryotrocha puerilis*, *Experientia* **7**, 222.

Bacci, G. (1951e) On two sexual races of *Asterina gibbosa*, *Experientia* **7**, 31.

Bacci, G. (1952a) Diverso comportamento sessuale delle *Ophryotrocha puerilis* di Napoli e di Plymouth, *Boll. Soc. It. Biol. Sper.* **28**, 1293.

Bacci, G. (1952b) Amputazione e successione delle fasi sessuali nel Polichete *Ophryotrocha puerilis*, *Boll. Soc. It. Biol. Sper.* **28**, 778.

Bacci, G. (1952c) Osservazioni citologiche sulla inversione sessuale nella *Patella vulgata* di Plymouth, *Boll. Zool.* **19**, 57–63.

Bacci, G. (1955a) La variabilità dei genotipi sessuali negli animali ermafroditi, *Pubbl. Staz. Zool. Napoli* **26**, 110–137.

Bacci, G. (1955b) Controllo genetico della inversione sessuale in individui isolati di *Ophryotrocha*, *Caryologia* (Suppl.), 966–968.

Bacci, G. (1956a) Indicazioni di un effetto materno sulla determinazione sessuale di *Dinophilus gyrociliatus*, *Ric. Scient.* **26** (Suppl.), 71–76.

Bacci, G. (1956b) Variabilità sessuale ed ermafroditismo, *Studi Sassaresi* **34**, 619–633.

Bacci, G. (1957) La variabilità sessuale nelle popolazioni eterogoniche, *Rend. Acc. Naz. Lincei* **23** (8), 165–173.

Bacci, G. (1961) Recenti ricerche sulla determinazione polifattoriale del sesso, *Boll. Zool.* **28**, 469–483.

Bacci, G. (1965) Sex determination and sex balance of *Ophryotrocha puerilis*. *Nature* (*Lond.*) **207**, 1208–1209.

Bacci, G., **Balata**, M. and **Romani**, M. L. (1958) Rapporti numerici dei sessi in tre popolazioni di *Mytilicola intestinalis* Steuer, *Rend. Acc. Naz. Lincei* **25**, 557–563.

Bacci, G. and **Bortesi**, O. (1960) Conferma della variabilità dei genotipi sessuali nel Polichete ermafrodita *Ophryotrocha puerilis siberti*, *Rend. Acc. Naz. Lincei* **28**, 92–94.

Bacci, G. and **Bortesi**, O. (1961a) Existence of multiple sex genotypes in the hermaphrodite Polychaete worm *Ophryotrocha puerilis siberti*, *Nature* (*Lond.*) **190**, 838.

Bacci, G. and **Bortesi**, O. (1961b) Pure males and females from hermaphroditic strains of *Ophryotrocha puerilis*, *Experientia* **17**, 229.

Bacci, G. and **la Greca**, M. (1953a) La differenziazione intraspecifica di *Ophryotrocha puerilis* nel Mediterraneo e nell'Atlantico, *Boll. Zool.* **20**, 93–98.

Bacci, G. and **la Greca**, M. (1953b) Genetic and morphological evidence for subspecific differences between Naples and Plymouth populations of *Ophryotrocha peurilis*, *Nature* (*Lond.*) **237**, 1208–1209.

Bacci, G. and **Pellegrini**, O. (1951) Successione delle fasi sessuali in una popolazione di *Calyptraea chinensis*, *Scientia Genetica*. **4**, 22–29.

Bacci, G. and **Razzauti**, A. (1957) Falso gonocorismo in *Coris julis*, *Rend. Acc. Naz. Lincei* **23** (8), 181–189.

Bacci, G. and **Razzauti**, A. (1958) Protogynous hermaphroditism in *Coris julis*, *Nature* (*Lond.*) **181**, 432–433.

Baltzer, F. (1914) Die Bestimmung des Geschlechts nebst einer Analyse des Geschlechtsdimorphismus bei *Bonellia*, *Mitt. Zool. Stat. Neapel* **22**, 1–44.

Baltzer, F. (1926) Über die Vermännlichung indifferenten *Bonellia* Larven durch *Bonellia* Exstrakte, *Rev. Suisse Zool.* **33**, 359–374.

Baltzer, F. (1932) Über die ohne Rüsselparasitismus entstehenden Spätmännchen (genetische Männchen) der *Bonellia viridis*, *Rev. Suisse Zool.* **39**, 281–305.

Baltzer, F. (1937) Entwicklungsmechanische Untersuchungen an *Bonellia viridis*. III. Ueber die Entwicklung und Bestimmung des Geschlechtes und die Anwendbarkeit des Goldschmidtschen Zeitgesetzes der Intersexualität bei *Bonellia viridis*, *Pubbl. Staz. Zool. Napoli* **16**, 89–159.

Bamber, R. C. (1927) Genetics of domestic cats, *Bibl. genet.* **3**, 1–83.

Bamber, R. C. (1925a) A thelytokous race of Cladocera in which pseudosexual reproduction occurs, *Z. ind. Abst. u. Vererbungsl.* **40**, 28–41.

Bamber, R. C. (1925b) The relations between previous sexual reproduction and the production of male offspring in *Moina macrocopa*, *Amer. Nat.* **59**, 50–61.

Barigozzi, C. (1951) Differenciation des génotype et distribution geographique d'*Artemia salina:* données et problèmes, *Année Biol.* **33,** 241–250.

Barr, M. L. (1955) The sex chromatin and its application to errors in sex development. *Modern Trends in Obstetrics and Gynecology,* Chapt. 7. London.

Barr, M. and Bertram, E. G. (1949) A morphological distinction between neurones of the male and female. *Nature* (*Lond.*) **163,** 676.

Bateson, W. and Punnett, R. C. (1911) The inheritance of the peculiar pigmentation of the silky fowl, *J. Genet.* **1,** 185-203.

Battaglia, B. (1958a) Selezione e rapporto dei sessi nel Copepode Harpacticoide *Tisbe gracilis, Rend. Acc. Naz. Lincei* **24** (Ser. 8), 327–335.

Battaglia, B. (1958b) Balanced polymorphism in *Tisbe reticulata,* a marine Copepod. *Evolution* **12,** 358–364.

Battaglia, B. (1959) Il polimorfismo adattativo e i fattori della selezione nel Copepode *Tisbe reticulata, Arch. Ocean. Limnol.* **12,** 145.

Battaglia, B. (1961) Rapporti fra geni per la pigmentazione e la sessualità in *Tisbe reticulata, Atti AGI* **6,** 139–447.

Battaglia, B. (1962) Gli effetti della selezione operata in assenza di inincrocio negli spostamenti del rapporto sessi in *Tisbe gracilis, Rend. Accad. Naz. Lincei* (8) **32,** 251–257.

Battaglia, B. (1963) Deviation from panmixia as a consequence of sex determination in the marine Copepod *Tisbe reticulata, Proc. XIth Int. Congr. Genetics* **1,** 9–26.

Battaglia, B. and Malesani, L. (1959) Ricerche sulla determinazione del sesso nel Copepode *Tisbe gracilis, Boll. Zool.* **26,** 423–433.

Beale, G. H. (1954) *The genetics of* Paramecium aurelia, Monographs in Experimental Biology, No. 2, Cambridge University Press.

Becak, W., Becak, M. L. and Nazareth, H. R. S. (1962) Karyotypic studies of two species of South American snakes. *Cytogenetics* **1,** 305–313.

Beccari, N. (1924) Studi sulla prima origine delle cellule genitali nei Vertebrati. III. Ricerche nel *Bufo viridis, Arch. Ital. Anat. Embr.* **21,** 332–374.

Beermann, W. (1955a) Cytologische Analyse eines *Camptochironomus* Artbastards. I. Kreuzungsergebnisse und die Evolution des Karyotypus, *Chromosoma* **7,** 198–259.

Beermann, W. (1955b) Geschlechtsbestimmung und Evolution der genetischen Y-Chromosomen bei *Chironomus, Biol. Zbl.* **74,** 525–544.

Beetschen, J. (1957) Relations entre les hypomorphoses et l'hétéroploidie consécutives à un choc hypothermique appliqué à l'oef fécondé du triton *Pleurodeles waltlii, C. R. Acad. Sci.* **244,** 2095–2097.

Belar, K. (1922) Untersuchungen an *Actinophrys sol.* I. Die Morphologie des Formwechsels, *Arch. Protistenk.* **46,** 1–96.

Belar, K. (1923) Über den Chromosomenzyclus von partenogenetischen Erdnematoden, *Biol. Zbl.* **43,** 513–518.

Belar, K. (1924) Die Cytologie der Merospermie bei freilebenden *Rhabditis* Arten, *Z. Zell. u. Gewebelehre.* **1,** 1–21.

Bellamy, A. W. (1922) Sex-linked inheritance in the Teleost *Platypoecilus maculatus, Anat. Rec.* **24,** 19–420.

Bellamy, A. W. (1936) Interspecific hybrids in *Platypoecilus:* one species ZZ-WZ; the other XY-XX, *Proc. Nat. Acad. Sci.* **22,** 531–536.

Bellamy, A. W. and Queal, M. L. (1951) Heterosomal inheritance and sex determination in *Platypoecilus maculatus, Genetics* **36,** 93–107.

Benazzi, M. (1946) Il sesso negli ibridi *Triton cristatus* ♀ × *Triton vulgaris* ♂, *Mon. Zool. Ital.* **55,** 132–137.

Benazzi, M. (1947) *Problemi biologici della sessualità,* Bologna, Cappelli.

Benazzi, M. (1956) Il sesso negli ibridi di tritoni e la questione della digametia sessuale negli urodeli, *Ricerca Scientif.* **26** (Suppl.), 1–11.

Benazzi, M. (1960) Ulteriori osservazioni sulla sessualità in ibridi del gen *Triturus, Atti AGI.* **5,** 191–197.

Bernstein, M. E. (1958) Studies in the human sex ratio. 5. A genetic explanation of the wartime increase in the secondary sex ratio, *Am. J. Human. Genet.* **10,** 68–70.

Berreur Bonnenfant, J. (1964) La culture in vitro d'organes de Crustacés, *Bull. Soc. Zool. France* **89**, 59–68.

Bishop, H. (1940) A study of sexuality in *Sapromyces reinschii*, *Mycologia* **32**, 505–529.

Blackburn, K. B. (1923) Sex chromosomes in plants, *Nature* **112**, 687–688.

Blacker, A. W. (1958) Contribution to the study of germ cells in the Anura, *J. Embryol. Exp. Morph.* **6**, 491–503.

Blakeslee, A. F. (1904) Sexual reproduction in the Mucorineae, *Proc. Amer. Acad. Sci.* **40**, 205–319.

Blakeslee, A. F. (1915) Sexual reactions between hermaphroditic and dioecious *Mucors*, *Biol. Bull.* **29**, 87–102.

Blochmann, F. (1889) Über die Richtungskörper bei den Eiern der Insekten, *Morph. Jahrb.* **12**.

Bompiani, A., de Carli, L., Moneta, E., Nuzzo, F. and Silvestrini, F. (1963) Un caso di mosaicismo XO XY, *Folia Hered. et Path.* **12**, 143–162.

Bonnemaison, L. (1951) Contribution a l'etude des facteurs provoquant l'apparition des formes ailées et sexuées chez les Aphidinae, *Ann. des epiphyties* **2**, 1–380.

Bonnemaison, L. (1958) Facteurs d'apparition des formes sexupares ou sexuées chez le Puceron cendré du Pommier (*Sappaphis plantaginea* Pass.), *Ann. Inst. nat. Rech. agron.* C, **9**, 329–353.

Bonoure, L. (1925) L'origine des gonocytes et l'evolution de la première ebauche génitale chez les Batraciens, *Ann. Sc. Nat.* **8**, 201.

Bonoure, L. (1937) La constitution des glandes génitales chez la Grenouille rousse après destruction étendue de la lignèe germinale. *C.R. Acad. Sci.* **204**, 1957.

Boveri, T. (1911) Über das Verhalten der Geschlechtschromosomen bei Hermaphroditismus. Boebachtungen an *Rhabditis nigrovenosa*, *Verh. phys. med. Ges. Würzb.* **41**, 83–97.

Boveri, T. (1915) Über die Entstehung der Eugsterschen Zwitterbienen, *Arch. Entwicklungs mech.* **41**.

Bowen, S. Th. (1963) The genetics of *Artemia salina*. II. White eye, a sex-linked mutation, *Biol. Boll.* **124**, 17–23.

Braverman, M. H. (1962) Studies in Hydroid differentiation. I, *Exp. Cell Res.* **26**, 301–306.

Breider, H. (1935a) Ueber Aussenfaktoren, die das Geschlechtsverhältniss bei *Xiphophorus helleri* kontrollieren sollen, *Z. wiss. Zool.* **146**, 383–413.

Breider, H. (1935b) Geschlechtsbestimmung u.—differenzierung bei *Limia nigrofasciata*, *caudofasciata*, *vittata* und deren Artbastarden, *Z. ind. Abst. u. Vererbungsl.* **68**, 265–299.

Breider, H. (1942) ZW-Männchen und WW-Weibchen bei *Platypoecilus maculatus*, *Biol. Zbl.* **62**, 187–195.

Breider, H. (1949) Der Geschlechtsbestimmungsmechanismus in der Artdifferenzierung, *Biol. Zbl.* **68**, 32.

Bridges, C. B. (1916) Non-disjunction as proof of the chromosome theory of heredity, *Genetics* **1**, 1–52, 107–153.

Bridges, C. B. (1921) Triploid intersexes in *Drosophila melanogaster*, *Science* **54**.

Bridges, C. B. (1925) Sex in relation to chromosomes and genes, *Amer. Nat.* **56**, 51–53.

Bridges, C. B. (1939) Cytological and genetical basis of sex. *Sex and Internal Secretions*, Ed. by Allen, I., Baltimore. pp. 15–63.

Brien, P. (1931) Le soma et le germen, *Ann. Bull. Soc. roy. Sci. med. nat. Bruxelles* 109–122.

Brien, P. and Reniers Decoen, M. (1949) La croissance, la blastogenése, l'ovogenese chez *Hydra fusca*, *Bull. Biol. France Belgique* **83**, 293–386.

Brink, J. M. von (1959) L'éxpression morphologique de la digametie chez les Sauropsidés, *Chromosoma* **10**, 1–71.

Brown, S. W. and Bennet, F. D. (1957) On sex determination in the diaspine scale *Pseudaulacaspis pentagona*, *Genetics* **42**, 510–523.

Buchner, H. (1941) Experimentelle Untersuchungen über den Generationswechsel der Rädertiere. II. *Zool. Jahrb.* (*Alleg. Zool.*) **60**, 279–343.

Buchner, H. and Mulzer, F. (1961) Untersuchungen über die Variabilität der Rädertiere. II. Der Ablauf der Variation im Freien, *Z. Morph. Ökol. Tiere* **50**, 330–374.

Buchner, P. (1954) Endosymbiosestudien an Schildläusen. I. *Stictococcus sjoestedti*, *Z. Morph. u. Ökol. Tiere* **43**, 262–312.

BULLER, A. H. R. (1930) The biological significance of conjugate nuclei in *Coprinus lagopus* and other Hymenomycetes, *Nature* **126**, 686–689.

BULLER, A. H. R. (1941) The diploid cell and the diploidization process in plants and animals with special reference to the higher fungi, *Bot. Rev.* **7**, 335–431.

BURGEFF, H. (1924) Untersuchungen über Sexualität und Parasitismus bei Mucorineae, *Bot. Abhandl.* **4**, 135.

BURKENROAD, M. O. (1937) The sex ratio in alternational hermaphrodites with special reference to the determination of sexual phases in oviparous oysters, *J. Mar. Res.* **1**, 75–84.

BURNS, R. K. (1925) The sex of parabiotic twins in Amphibia, *J. exp. Zool.* **42**, 31–90.

BURNS, R. K. (1935) The process of sex transformation in parabiotic *Amblystoma*. 3. *Anat. Rec.* **63**: 101–129.

BURNS, R. K. (1938) The effects of chrystalline sex hormones on sex differentiation in *Amblystoma*. I. Estrone, *Anat. Rec.* **71**, 447.

BUTTNER, A. (1950) Labilité particuliere du sexe chez *Schistosoma mansoni*. Essai d'interpretation, *Ann. Parasitol. Paris* **25**, 297–307.

CALLAN, H. G. (1941) Determination of sex in *Scalpellum*, *Nature* **148**, 258.

CAMENZIND, R. (1962) Untersuchungen über die bisexuelle Fortpflanzung einer pädogenetischen Gallmücke, *Rev. Suisse Zool.* **69**, 377–384

CANELLA, M. (1959) Biologia degli Infusori e ipotetici raffronti con i Metazoi. Problematica dell'anfimissi. II, *Mon. Zool. Ital.* **66**, 198–228.

CARR, D. H., BARR, M. L. and PLUNKETT, E. R. (1961) A probable XXYY sex determining mechanism in a mentally defective male with Klinefelter's syndrome. *Canad. med. Ass. J.* **84**, 873–878.

CASTLE, W. E. (1903) The heredity of sex. *Bull. Mus. Comp. Zool.* **40**, 4.

CATTANACH, B. M. (1961) XXY mice. *Genet. Res.* **2**, 156–158.

CAULLERY, M. (1913) *Les problémes de la sexualité*. Paris, Flammarion.

CAULLERY, M. and COMAS, M. (1928) Le determinisme du sexe chez un Nematode (*Paramermis contorta*) parasite des larves de *Chironomes*, *C. R. Acad. Sci.* **186**, 664.

CAVALCANTI, A. G. L., FALCAO, D. N. and CASTRO, L. E. (1957) Sex ratio in *Drosophila prosaltans*, a character due to interaction between nuclear genes and cytoplasmic factors, *Am. Naturalist* **91**, 327–329.

CAVALLI, L. L., LEDERBERG, G. and LEDERBERG, E. M. (1953) An infective factor controlling sex compatibility in *Bacterium coli*, *J. Gen. Microb.* **8**, 89.

CHARNIAUX, H. (1952) Castration chirurgicale chez un Crustacè Amphipode (*Orchestia gammarella*) et déterminisme des caractères sexuels secondaires, *C. R. Acad. Sci.* **234**, 2570–2572.

CHARNIAUX COTTON, H. (1954) Découverte chez un Crustacè Amphipode d'une glande endocrine responsable de la differenciation des caractères sexuels primaires et secondaires mâles. *C. R. Acad. Sci.* **240**, 1487–1489.

CHARNIAUX COTTON, H. (1956) Déterminisme hormonal de la différenciation sexuelle chez les Crustacés, *Année Biol.* **32**, 371–399.

CHARNIAUX COTTON, H. (1957a) Croissance, regeneration et déterminisme endocrinien des caractères sexuels d'*Orchestia gammarella*, *Ann. Sc. Nat. Zool.* **19**, 411–559.

CHARNIAUX COTTON, H. (1957b) Déterminisme des phenoménes d'intersexualitè chez *Orchestia gammarella*. Première résultats, *C. R. Acad. Sci.* **245**, 1665–1668.

CHARNIAUX, COTTON H. (1958b) Contrôle hormonale de la différenciation du sexe et de la reproduction chez les Crustacés supérieurs, *Bull. Soc. Zool. Fr.* **83**, 314–336.

CHARNIAUX COTTON, H. (1958c) La glande androgène de quelques Crustacés decapodes et particulièrement de *Lysmata seticaudata*, *C. R. Acad. Sci.* **246**, 2814–2817.

CHARNIAUX COTTON, H. (1960) Physiologie de l'inversion sexuelle chez la crevette à hermaphrodisme protërandrique fonctionel, *Lysmata seticaudata*, *C. R. Acad. Sci.* **250**, 4046–4048.

CHARNIAUX COTTON, H. (1961a) La croissance et la morphogenèse des caractères sexuels des Crustacés supèrieurs et l'hormone androgène, *Bull. Soc. Zool. France* **86**, 484–499.

CHARNIAUX COTTON, H. (1961b) Physiologie de la gonade de *Lysmata seticaudata* chez les individus normaux et les femelles masculinisées, *C. R. Acad. Sci.* **252**, 199–201.

Charniaux Cotton, H. (1962) Androgenic gland of the Crustaceans, *Gen. Comp. Endocrin.* (Suppl.) **1,** 241–247.

Charniaux Cotton, H. (1963) Démonstration expérimentale de la secrétion d'hormone femelle par le testicule inversé en ovaire de *Talitrus saltator*, *C. R. Acad. Sci.* **256,** 4088–4091.

Charniaux Cotton, H. (1964) Endocrinologie et génétique du sexe chez les Crustacés supérieurs, *Ann. Endocrinologie* **25,** 36–42.

Chiarelli, B. (1961) Chromosomes of the orang utan, *Nature* **192,** 285.

Chiarelli, B. (1962) Comparative morphometric analysis of primate chromosomes. I. The chromosomes of apes and man, *Caryologia* **15,** 99–121.

Chieffi, G. (1949) Ricerche sul differenziamento dei sessi negli embrioni di *Torpedo ocellata*, *Pubbl. Staz. Zool. Napoli.* **22,** 57–78.

Chieffi, G. (1952) Sull'organogenesi dell'interrenale e della medulla della gonade in *Torpedo ocellata* e *Scylliorhinus canicula*, *Pubbl. Staz. Zool. Napoli* **23,** 185.

Chieffi, G. (1955) Nuove osservazioni nell'organogenesi della medulla della gonade dei Vertebrati. Ricerche istochimiche in *Rana esculenta*, *Bufo viridis* e *Scylliorhinus caniculata*, *Pubbl. Staz. Zool. Napoli* **27,** 62.

Chieffi, G. (1957) Nuove osservazioni sull'organogenesi della medulla della gonade dei Vertebrati, *Pubbl. Staz. Zool. Napoli* **27,** 62–72.

Cleveland, L. R. (1949) Hormone induced sexual cycles of Flagellates. I. Gametogenesis, fertilization, and meiosis in *Trichonympha*, *J. Morph.* **85,** 197–296.

Cleveland, L. R. (1950) Hormone induced sexual cycles of Flagellates. IV. Meiosis after syngamy and before nuclear fusion in *Notila*, *J. Morph.* **87,** 317–348.

Cleveland, L. R. (1956) Cell division without chromatin in *Trichonympha* and *Barbulanympha*, *J. Protozool.* **3,** 78–83.

Cleveland, L. R. (1957) Correlation between the molting period of *Cryptocercus* and sexuality in its protozoa, *J. Protozool.* **4,** 168–175.

Cleveland, L. R. (1959) Sex induced with ecdysone, *Proc. Nat. Acad. Sci.* **45,** 747–753.

Cleveland, L. R., Hall, S. R., Sandres, E. P. and Collier, J. (1934) The wood-feeding roach *Cryptocercus*, its protozoa, and the symbiosis between protozoa and roach, *Mem. Am. Acad. Arts Sci.* **17,** 185–342.

Cleveland, L. R. and Nutting, W. L. (1956) Suppression of sexual cycles and death of the protozoa of *Cryptocercus* resulting from change of hosts during molting period, *J. Exper. Zool.* **130,** 485–514.

Coe, W. R. (1932) Sexual phases in the American oyster (*Ostrea virginica*), *Biol. Bull.* **63,** 419–441.

Coe, W. R. (1943) Sexual differentiation in Molluscs. I. Pelecypods, *Quart. Rev. Biol.* **18,** 154.

Coe, W. R. (1944) Sexual differentiation in Molluscs. II. Gastropods, Amphineurians, Scaphopods and Cephalopods, *Quart. Rev. Biol.* **19,** 85.

Coe, W. R. (1948) Variations in the expression of sexuality in the normally protandric gastropod *Crepidula plana*, *J. Exper. Zool.* **108,** 155–170.

Cognetti, G. (1954) La proteroginia in una popolazione di *Asterina pancerii* del Golfo di Napoli, *Boll. Zool.* **21,** 77–80.

Cognetti, G. (1956a) Autofecondazione in *Asterina*, *Boll. Zool.* **23,** 275–278.

Cognetti, G. (1956b) Controllo genetico dell'inizio della stolonizzazione in alcune specie di *Autolytus*, *Ricerca Scientifica.* **26,** (Suppl.), 141–144.

Cognetti, G. (1961a) Endomeiosis in parthenogenetic lines of Aphids, *Experientia* **17,** 168.

Cognetti, G. (1961b) Citogenetica della partenogenesi negli Afidi, *Arch. Zool. It.* **46,** 89–122.

Cognetti, G. and Delavault, R. (1960) Gonocorismo stabile, gonocorismo labile ed ermafroditismo in Asteroidi dell'Atlantico e del Mediterraneo, *Rend. Acc. Naz. Lincei* **28,** 82–85.

Cognetti, G. and Delavault, R. (1962) La sexualité des Astérides, *Cah. Biol. Mar.* 3, 157–182.

Cognetti, G. and Pagliai, A. M. (1963) Razze sessuali in *Brevicoryne brassicae*, *Arch. Zool. Ital.* **48,** 329–337.

COLOMBO, M. (1957) On the sex ratio in man, *Cold Spring Harbour Symp.* **22,** 193–202.

COMAS, M. (1927) Sur l'intersexualité de *Paramermis contorta*, *Bull. Biol. Fr. Belgique* **61,** 186–189.

COMMONER, B. (1964) Roles of deoxiribonucleic acid in inheritance, *Nature* **202,** 960–968.

COOPER, K. W. (1949) The cytogenetics of meiosis in *Drosophila*. Mitotic and meiotic autosomal chiasmata without crossing-over in the male, *J. Morph.* **84,** 81–122.

CORRENS, C. (1907) *Die Bestimmung und Vererbung des Geschlechts, nach neuen Versuchen mit höheren Pflanzen.* Berlin.

CORRENS, C. (1928) Bestimmung, Vererbung und Verteilung des Geschlechts bei den höheren Pflanzen, *Handb. d. Vererbungswiss.* **2,** 1–138.

COUCH, J. N. (1926) Heterothallism in *Dictyuchus*, a genus of the water moulds, *Ann. Bot.* **40,** 849–881.

CREW, F. (1921) Sex reversal in frogs and toads, *J. Genet.* **11.**

CREW, F. (1923) Complete sex transformation in the domestic fowl, *J. Hered.* **14,** 360–362.

CREW, F. (1937) The sex ratio, *Nature* (*Lond.*) **140,** 449–453.

CROUSE, H. V. (1943) Translocations in *Sciara:* their bearing on chromosome behaviour and sex determination, *Missouri Univ. Research Bull.* **379,** 1–75.

CROUSE, H. V. (1960a) The nature of the influence of X translocations on sex of progeny in *Sciara coprophila*, *Chromosoma* **11,** 146–166.

CROUSE, H. V. (1960b) The controlling element in the sex chromosome behaviour in *Sciara*, *Genetics* **45,** 1429–1443.

CUÉNOT, L. (1898) Notes sur les Echinodermes. III. L'hermaphroditisme protandrique d'*Asterina gibbosa* et ses variations suivant les localités, *Zool. Anz.* **21,** 273–279.

DAHLBERG, G. (1951) The primary sex ratio and its ratio at birth, *Acta genet.* **2,** 245–251.

DALMON, J. (1938) Changement de sexe et hermaphroditisme transitoire chez *Chlamys varia*, *C. R. Acad. Sci.* **207,** 181–183.

D'ANCONA, U. (1941) Ulteriori osservazioni e considerazioni sull'ermafroditismo e il differenziamento sessuale dell'orata (*Sparus auratus*), *Pubbl. Staz. Zool. Napoli* **18,** 313–336.

D'ANCONA, U. (1946) Ermafroditismo e gonocorismo in alcuni Sparidi, *Boll. Soc. Ital. Biol. Sper.* **22,** 2.

D'ANCONA, U. (1949) Ermafroditismo e intersessualità nei Teleostei, *Experientia.* **5,** 381–389.

D'ANCONA, U. (1951) Il differenziamento della gonade e l'inversione sessuale degli Sparidi, *Arch. Ocean. Limn.* **6,** 97–163.

DANFORTH, C. H. (1936) Relation of genic and endocrine factors in sex. *Sex and internal secretion* Ed. by Allen. Baltimore.

DANTCHAKOFF, V. (1935) Sur l'inversion sexuelle expérimentale de l'ébauche testiculaire chez l'embryon de poulet, *C. R. Acad. Sci.* **200.**

DANTCHAKOFF, V. (1941) *Der Aufbau des Geschlechts beim höheren Wirbeltier.* Jena, Fischer.

DARLINGTON, C. D. (1931) Meiosis, *Biol. Rew.* **6,** 221–264.

DARLINGTON, C. D. (1932) *Recent advances in cytology*, London.

DARLINGTON, C. D. (1956) *Chromosome Botany*, Allen & Unwin, London.

DARLINGTON, C. D. (1958) *Evolution of genetic systems*, 2nd. edn. London, Cambridge University Press.

DARLINGTON, C. D. and DOBZHANSKY, T. (1942) Temperature and sex ratio in *Drosophila pseudoobscura*, *Proc. Nat. Acad. Sci.* **28,** 45–47.

DEHN, M. VON (1937) Experimentelle Untersuchungen über den Generationswechsel der Cladoceran, *Zool. Jahrb.* (*Phys.*) **58,** 241–272.

DEHN, M. VON (1949) Experimentelle Untersuchungen über den Generationswechsel der Cladoceren. II. Cytologische Untersuchungen bei *Moina rectirostris*, *Chromosoma.* **3,** 167–194.

DEHN, M. VON (1950) Der Einfluss der Hefsfettstoffe auf die Fortplanzungsart von *Moina rectirostris*, *Naturwiss.* **37,** 429–430.

DEHN, M. VON (1955) Die Geschlechtsbestimmung der Daphniden. Die Bedeutung der Fettstoffe untersucht an *Moina rectirostris*, *Zool. Jahrb.* **65**, 334–356.

DE LATTIN, G. (1949) Ein Farbgen als relativer Geschlechtsrealisator bei *Porcellio scaber*, *Naturwiss.* **36**, 89.

DE LATTIN, G. (1951) Über die Bestimmung und Vererbung des Geschlechts einiger Oniscoiden. 1. Untersuchungen über die Geschlechtsbeeinflussende Wirkung von Farbfaktoren bei *Porcellio* und *Tracheoniscus*, *Z. ind. Abst. u. Vererbungsl.* **84**, 1–37.

DE LATTIN, G. (1952) Über die Bestimmung und Vererbung des Geschlechts einiger Oniscoiden. II. Zur Vererbung der Monogenie von *Cylisticus convexus*, *Z. ind. Abst. u. Vererbungsl.* **84**, 536–567.

DE LATTIN, G. (1958) Vererbungsstudien an Isopoden. I. Zur Genetic der Farbvariabilität von *Cylisticus convexus*, *Zool. Anz.* **160**, 313–324.

DE LATTIN, G. and GROW, F. J. (1953) Die Beinflussbarkeit sekundärer Geschlechtsmerkmale von *Oniscus asellus* duerch die Gonaden, *Experientia* **9**, 338.

DELAVAULT, R. (1957) Croissance des ovotestis puis des ovaires chez un Nématode libre hermaphrodite: *Caenorhabditis elegans*, *Bull. Soc. Zool. Fr.* **82**, 321–325.

DELAVAULT, R. (1958) L'autofécondation chez les métazoires, *Année Biol.* (Sér. 3) **34**, 5–16.

DELAVAULT, R. (1960) Les cycles génitaux chez *Asterina gibbosa* de Dinard, *C. R. Acad. Sci.* **251**, 2240–2241.

DEROUX, G. (1960) Formation réguliére de mâles mûrs, de taille et d'organisation larvaire chez un Eulamellibranche commensal (*Montacuta phascolionis*), *C. R. Acad. Sci.* **250**, 2264–2266.

DOBELL, C. C. (1925) The life history and chromosome cycle of *Aggregata eberthi*, *Parasitology.* **17**, 1–136.

DOBZHANSKY, TH. (1935) Some remarks on Goldschmidt's critique of the hypothesis of multiple sex genes, *J. Genet.* **31**, 155–156.

DOBZHANSKY, TH. (1939) Fatti e problemi della condizione "rapporto sessi" (sex ratio) in *Drosophila*, *Scientia Genetica* **1**, 67–75.

DOBZHANSKY, T. and SCHULTZ, J. (1931) Evidence for multiple sex factors in the X chromosome of *Drosophila melanogaster*, *Proc. Nat. Acad. Sci.* **17**, 513–518.

DOBZHANSKY, T. and SCHULTZ, J. (1934) The distribution of sex factors in the X chromosome of *Drosophila melanogaster*, *J. Genet.* **28**, 349–385.

DOBZHANSKY, T. and SPASSY, B. (1941) Intersexes in *Drosophila pseudoobscura*, *Proc. Nat. Acad. Sci.* **27**, 556–562.

DODD, J. M. (1956) Studies in the biology of limpets. III. Hermaphroditism in three British species of *Patella*, *J. Mar. Biol. Ass.* **35**, 327–340.

DODDS, K. S. (1939) Oogenesis in *Neuroterus baccarum*, *Genetics* **21**, 177–190.

DODGE, B. O. (1932) The non sexual and sexual function of microconidia of *Neurospora*, *Bull. Torrey Botan. Club.* **59**, 347–360.

DOGIEL, V. (1925) Die Geschlechtsprozesse bei Infusorien, speziell den Ophryoscoleciden, *Arch. Protistenk.* **50**, 283–442.

DONCASTER, L. (1910) Gametogenesis of the gall-fly *Neuroterus lenticularis*. I, *Proc. Roy. Soc.* B. **83**, 476–489.

DONCASTER, L. (1916) Gametogenesis and sex determination of the gall-fly. III, *Proc. Roy. Soc.* B. **89**, 183–200.

DONCASTER, L. and RAYNER, G. H. (1906) On breeding experiments with Lepidoptera, *Proc. Zool. Soc.* 125–133.

DOUIN, C. (1909) Nouvelles observations sur *Sphaerocarpus*, *Rev. Bryol.* **36**.

DRACH, P. (1939) Mue et cycle d'intermue chez les Crustaces Decapodes, *Ann. Inst. Ocean.* **19**, 103–391.

DRESCHER W. and ROTHENBUHLER, W. C. (1964) Sex determination in the honey bee, *J. Hered.* **55**, 91–96.

DREYFUS, A. (1937) Contribuçao para o estudio do ciclo cromosomico e da determinaçao do sexo de *Rhabdias filleborni*, *Bol. Fac. Filos. Cienc. S. Paulo, Biol. Ger.* **1**, 1–144.

DREYFUS, A. and BREUER, M. E. (1944) Chromosomes and sex determination in the parasitic Hymenopteron *Telenomus fariai*, *Genetics* **29**, 75–82.

DROMAMRAYU, K. R. (1961) Hypertrichosis of the pinna of the human ear, Y linked pedigrees, *J. Genet.* **57**, 230–241.

DROMAMRAYU, K. R. (1964) Y linkage in man, *Nature* (*Lond.*) **201**, 424–425.

DURCHON, M. (1951) Stolonisation et hermaphroditisme successif chez *Syllis amica*, *Arch. Zool. Expér. Gen.* **88**, 96–100.

DURCHON, M. (1958) Potentialités de sexualisation réelles et virtuelles au cours de la stolonisation chez les Syllinae, *C. R. Acad. Sci.* **246**, 2297–2298.

DURCHON, M. (1959) Contribution a l'étude de la stolonisation chez les Syllidiens: I. Syllinae, *Bull. Biol. Fr. Belgique*. **93**, 156–219.

EDMONDSON, W. T. (1955) The seasonal life history of *Daphnia* in an Arctic lake, *Ecology* **30**, 439–455.

EGAMI, N. (1951) A note on the sex differentiation of the marine Copepod *Tigriopus japonicus*, *Annot. Zool. Japon.* **24**, 131–136.

ELLIOT, A. M. (1959) A quarter century exploring *Tetrahymena*, *J. Protozool.* **6**, 1–7.

EMERSON, R. A. (1932) The present status of Maize genetics, *Proc. VI. Int. Congr. Gen.* 141–152.

FALCONER, D. S. (1952) A totally sex-linked gene in the house mouse, *Nature* (*Lond.*) **169**, 664–665.

FERGUSON SMITH, M. A. (1964) The sites of nucleolus formation in human pachytene chromosomes, *Cytogenetics*, **3**, 124–134.

FILIPPONI, A. (1955) Un nuovo caso di arrenotochia nei Macroclielidi (Acarina, Mesostigmata), *Riv. Parassit.* **16**, 145–168.

FILIPPONI, A. and SEGANTI, L. (1957) Arrenotochia in *Macrocheles subbadius* (Acarina, Mesostigmina), *Riv. Parassit.* **18**, 27–33.

FORD, C. E. and HAMERTON, J. L. (1956) The chromosomes of man, *Nature* (*Lond.*) **178**, 1020–1023.

FORD, C. E., JONES, K. W., MILLER, O. J., MITTWOCH, U., PENROSE, L. S., RIDLER, M. and SHAPIRO, A. (1959) The chromosomes in a patient showing both mongolism and the Klinefelter syndrome, *Lancet*. **1**, 709–710.

FORD, C. E., JONES, K. W., POLANI, P. E., DE AMEIDA, J. C. and BRIGGS, J. H. (1959) A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome), *Lancet*. **1**, 711–713.

FORD, C. E., POLANI, P. E., BRIGGS, J. H. and BISHOF, P. M. F. (1959) A presumptive XXY/XX mosaic, *Nature* (*Lond.*) **183**, 1030–1032.

FORSTER, H. (1957) Die Wirkungsspektrum der Population von *Chlamydomonas eugametos*, *Z. Naturforsch.* **12**b, 765–770.

FORSTER, H., WIESE, L. and BRAUNITZER, G. (1956) Über das agglutinierend wirkende Gynogamon von *Chlamydomonas eugametos*, *Z. Naturforsch.* **11**b, 315–317.

FØYN, B. (1950) Sex and Inheritance in the Serpulid *Pomatoceros triqueter*, *Nature* (*Lond.*) **165**, 652–653.

FRACCARO, M., BOTT, M., SALZANO, F. M., ROSS RUSSELL, R. W. and CRAUSTON, W. I. (1962) Triple chromosomal mosaic in a woman with clinical evidence of masculinization, *Lancet*. i, 1379–1381.

FRACCARO, M., DAVIES, P., BOTT, M. G. and SCHUTT, W. (1962) Mental deficiency and undescended testis in two males with XYY sex chromosomes, *Folia hered. path, Milano* **11**, 211–220.

FRACCARO, M., KLINGER, H. P. and SCHUTT, W. (1962) A male with XXXXY sex chromosomes, *Cytogenetics* **1**, 52–64.

FRASER, J. H., BOYD, E., LENNOX, B. and DENNISON, W. M. (1961) A case of XXXXY Klinefelter's syndrome, *Lancet* ii, 1064–1067.

FRIES, G. (1964) Über die Einwirkung der Tagesperiodik und der Temperatur auf den Generations -wechsel, die Weibchengrosse und die Eier von *Daphnia magna*, *Z. morph. Ökol. Tiere* **53**, 475–516.

GABE, D. R. (1939) Inheritance of sex in *Mercurialis annua*, *Compt. rend. acad. sci. U.R.S.S.* **23**, 478–481.

Gairdner, A. E. (1933) Sporangia containing spermatozoids in Ferns, *Nature* (*Lond.*) **131**, 621.

Gálán, F. (1946) Sur la génétique de la monoecie et la dioecie zygotiques chez *Ecballium elaterium* Rich, *C. R. Acad. Sci.* **222**, 1130.

Gálán, F. (1950) Analyse génétique de la monoecie et de la dioecie zygotiques et de leur différence dans *Ecballium elaterium*, *Proc. 7th Intern. Botan. Congr. Stockholm*. p. 340.

Gálán, F. (1951) Analyse génétique de la monoecie et de la dioecie zygotiques et de leur différence dans *Ecballium elaterium*, *Acta Salmanticensia, Ciencias: Sec. Biol.* **1**, 7–15.

Galgano, M. (1933a) Studi intorno al comportamento della cromatina nella spermatogenesi di *Rana esculenta*. Il numero e la forma dei cromosomi nel processo normale, *Arch. Ital. Anat. Embriol.* **31**.

Galgano, M. (1933b) Evoluzione dei spermatociti di I ordine e cromosomi pseudosessuali in alcune specie di anfibi, *Arch. Ital. Anat. Embroil.* **32**, 171–200.

Galgano, M. (1940) Prime ricerche intorno alla fisiologia del ciclo sessuale maschile di *Triton cristatus*. Influenza dell'ipofisi sopra alcuni caratteri sessuali dei castrati, *Mon. Zool. Ital.* **51**, 207–218.

Galgano, M. (1944) Il ciclo sessuale annuale in *Triturus cristatus carnifex* (Laur.). I. Il ciclo naturale nei due sessi, *Arch. Ital. Anat. Embriol.* **50**, 1–148.

Galgano, M. (1947) Il ciclo sessuale annuale in *Triturus cristatus carnifex* (Laur.). II. Azione della temperatura e di altri fattori ambientali sul ciclo maschile e femminile, *Arch. Zool. Ital.* **32**, 33–207.

Gallien, L. (1937) Action masculinisante du propionate de testostérone dans la differenciation du sexe, chez *Rana temporaria*, *C. R. Acad. Sci.* 20–375.

Gallien, L. (1940) Rôle du solvent et de la méthode d'administration de la dihydrofolliculine dans la différenciation du sexe chez les Batraciens, *C. R. Acad. Sci. Paris* **211**, 570.

Gallien, L. (1954) Inversion expérimentale du sexe sous l'action des hormones sexuelle chez le Triton, *Pleurodeles waltlii*, *Bull. Biol. Fr. Belgique* **88**, 1.

Gallien, L. (1956) Inversion expérimentale du sexe chez un Anoure inférieur *Xenopus laevis*. Analyse des conséquences génétiques, *Bull. Biol. Fr. Belgique* **90**, 163–183.

Gallien, L. (1963) Différenciation sexuelle d'individus haploïdes du Triton *Pleurodeles waltlii*, mis en parabiose. *C. R. Acad. Sci.* **257**: 2890–2893.

Galun, E. (1961) Study of the inheritance of the sex expression in the cucumber, *Genetica* **32**, 134–163.

Galun, E., Yung, Y. and Lang, H. (1962) Culture and sex modification of male cucumber buds in vitro, *Nature* (*Lond.*) **194**, 596–598.

Gaudenzi, R. and Zunarelli, R. (1960) Influenza di fattori ambientali nella produzione di femmine mittiche e di maschi in due specie di *Brachionus*, *Arch. Zool. Ital.* **45**, 159–173.

German, J. L. (1962) DNA synthesis in human chromosomes, *Trans. N.Y. Acad. Sci.* **24**, 395–407.

Gershenson, S. (1928) A new sex ratio abnormality in *Drosophila obscura*. *Genetics*, **13**, 488–507.

Ghirardelli, E. (1961) Risultati di esperimenti di trapianto nella regione posteriore dell' abbozzo genitale in embrioni di *Bufo bufo*. *Rendic. Accad. Naz. Lincei* **31**, 152–157.

Ghirardelli, E. (1961a) Alcuni risultati di esperimenti di asportazione e di trapianto nella regione genitale di embrioni di *Bufo*. *Rend. Accad. Naz. Lincei* **30**, 284–291.

Ghirardelli, E. (1962) Nuovi risultati di esperimenti di trapianto nella regione anteriore dell'abbozzo genitale in embrioni di *Bufo bufo*. *Rendic. Accad. Naz. Lincei* **31**, 485–489.

Giard, A. (1887) La castration parasitaire et son influence sur les caractéres extérieures du sexe male chez les Crustacés Décapodes. *Bull. Sci. Dép. du Nord.*, **18**, 1–28.

Gilchrist, B. M. and Haldane, J. B. S. (1947) Sex linkage and sex determination in a mosquito, *Culex molestus*. *Hereditas*, **33**, 175–190.

Goldschmidt, R. (1912) Erblichkeitstudien an Schmetterlingen. *Z. ind. Abst. u. Vererbungsl.*, **7**, 1–62.

Goldschmidt, R. (1915) Vorläufige Mitteilung über weiteren Versuche zur Vererbung und Bestimmung des Geschlechts. *Biol. Zbl.*, **35**, 565–570.

Goldschmidt, R. (1920) Mechanisms und Physiologie der Geschlechts bestimmung. *Berlin, Bornträger.*

Goldschmidt, R. (1923) Untersuchungen über Intersexualität. III. *Z. ind. Abst. u. Vererbungsl.* **31**, 100–133.

Goldschmidt, R. (1929) Geschlechtsbestimmung im Tier-und Pflanzenreich. *Biol. Zbl.*, **49**, 641–648.

Goldschmidt, R. (1931) Die sexuelle Zwischenstufen. *Springer, Berlin.*

Goldschmidt, R. (1935) Multiple sex genes in *Drosophila*? A critique. *J. Genet.*, **31**, 145–153.

Goldschmidt, R. (1938) Physiological genetics. *New York.*

Goldschmidt, R. (1942) Sex determination in *Melandrium* and *Lymantria. Science*, **95**, 120–121.

Goldschmidt, R. (1955) Theoretical Genetics. *Univ. of California Press. Berkeley.*

Goldschmidt, R. and Katsuchi, K. (1927) Erblicher Gynandromorphismus und somatische Mosaicbildung bei *Bombyx mori*, *Biol. Zbl.* **47**, 45–55.

Goldschmidt, R. and Katsuchi, K. (1928) Zweite Mitteilung über Erblicher gynandromorphismus bei *Bombyx mori*, *Biol. Zbl.* **48**, 39–43.

Gordon, M. (1937) Genetics of Platypoecilus. III. Inheritance of sex and crossing-over of the sex chromosomes of the platyfish, *Genetics* **22**, 376–392.

Gordon, M. (1946) Interchanging genetic mechanisms for sex determination in fishes under domestication, *J. Hered.* **37**, 307–320.

Gordon, M. (1947) Genetics of *Platypoecilus maculatus*. IV.The sex determining mechanism in two wild populations of the Mexican platyfish, *Genetics* **32**, 8–17.

Gordon, M. (1951) Genetics of *Platypoecilus maculatus*. V. Heterogametic sex determining mechanisms in females of a domesticated stock originally from British Honduras, *Zoologica* (*N.Y.*) **37**, 91–100.

Gordon, M. (1952) Sex determination in *Xiphophorus maculatus*. III. Differentiation of gonads from broods having a sex ratio of three females to one male, *Zoologica* (*N.Y.*) **37**, 91–100.

Gordon, M. (1957) Physiological genetics of fishes. *The Physiology of Fishes*. Ed. by Brown, Vol. 2. New York. p. 431–501.

Gordon, M. (1957) Control of sex ratio in rabbits by electrophoresis of spermatozoa, *Proc. Nat. Acad. Sci.* **43**, 913–918.

Gould, H. N. (1917) Studies on sex in the hermaphrodite Mollusk *Crepidula plana*. 2. Influence of environment on sex, *J. Exper. Zool.* **23**, 225–250.

Gould, H. N. (1919) Studies on sex in the hermaphrodite Mollusk *Crepidula plana*. 3. Transference of the male producing stimules through sea water, *J. Exper. Zool.* **29**, 113–120.

Gowen, J. W. (1942) On the genetic basis for hermaphroditism, *Anat. Rec.* **84**, 458.

Gowen, J. W. and Fung Sui Tong Chan (1957) Determination of sex through genes in a major sex locus in *Drosophila melanogaster*. *Heredity*. **11**, 397–402.

Granata, L. (1909) Le divisioni degli spermatociti di *Xylocopa violacea*, *Biologica* **2**, 1–12.

Grumbach, M. M., Morishima, A. and Taylor, J. H. (1963) Human sex chromosome abnormalities in relation to DNA replication and heterochromatinization, *Proc. Nat. Acad. Sci.* **49**, 581–589.

Haldane, J. B. S. (1922) Sex ratio and unisexual sterility in hybrid animals, *J. Genet.* **12**, 101–109.

Haldane, J. B. S. (1931) *The Causes of Evolution*. London.

Haldane, J. B. S. (1936) A search for incomplete sex linkage in man, *Ann. Eugen.* (*Lond.*) **7**, 28–57.

Haldane, J. B. S. (1955) Some alternatives to sex, *New Biology*, **19**, 7–26.

Hamerton, L. J., Fraccaro, M., de Carli, L. *et al.* (1961) The somatic chromosomes of the *Gorilla*, *Nature* (*Lond.*) **192**, 225–228.

Hámmerling, J. (1937) Fortpflanzung und Sexualität, *Fortschr. d. Zool.*, *N.F.* **4**, 467–500.

Hámmerling, J. (1947) Fortpflanzung und Sexualität, *Fortschr. d. Zool. N.F.* **8**, 159–188.

Harms, J. W. (1921) Verwandlung des Bidderschen Organs in ein Ovarium beim Männchen von *Bufo vulgaris*, *Zool. Anz.* **53**, 253–265.

Hartmann, M. (1904) Die Fortpflanzungsweise der Organismen, Neubenennung und Einteilung, *Biol. Zbl.* **24**, 18–33.

Hartmann, M. (1909) Autogamie bei Protisten und ihre Bedeutung für das Befruchtungsproblem. *Arch. Protistenk.* **14**, 264–284.

Hartmann M. (1925) Über relative Sexualität bei *Ectocarpus siliculosus*. Ein experimenteller Beitrag zur Sexualitätshypothese der Befruchtung, *Naturwiss.* **13**. 975–980.

Hartmann, M. (1929) Fortpflanzung und Befruchtung als Grundlage der Vererbung. *Handb. d. Vererbungswissensch.*, *Lief.* **6**, 1–103.

Hartmann, M. (1930) Die Sexualität der Protisten und Tallophyten und ihre Bedeutung für eine allgemeine Sexualitätstheorie, *Z. ind. Abst. u. Vererbungsl.* **54**, 76–126.

Hartmann, M. (1931) Relative Sexualität und ihre Bedeutung für eine allegemeine Sexulitäts und Befruchtungstheorie, *Naturwiss.* **19**, 230–232.

Hartmann, M. (1932) Neue Ergebnisse zum Befruchtungs-und Sexualitätsproblem, *Naturwiss.* **20**, 567–573.

Hartmann, M. (1934) Untersuchungen über die Sexualität von *Ectocarpus siliculosus*, *Arch. Protistenk.* **83**, 110–153.

Hartmann, M. (1937) Ergänzende Untersuchungen über die Sexualität von *Ectocarpus silicolosus*, *Arch. Protistenk.* **89**, 382–392.

Hartmann, M. (1943) *Die Sexualität*, 1st ed. Fischer, Jena.

Hartmann, M. (1956) *Die Sexualität*, 2nd ed. Fischer, Stuttgart.

Hartmann, M. and Huth, W. (1936) Untersuchungen u. Geschlechtsbestimmung u. Geschlechtsumwandlung von *Ophryotrocha puerilis*, *Zool. Jahrb.* **56**, 389–439.

Hartmann, M. and Lewinski, G. (1938) Untersuchungen. . . . II. Versuche über die Wirkung von Kalium, Magnesium u. Kupfer, *Zool. Jahrb.* (*Phys.*) **58**, 551–574.

Hartmann, M. and Lewinski, G. (1940) Untersuchungen . . . . III. Die stoffliche Natur der vermännlichenden Wirkung "stärker" Weibchen (Eistoffe), *Zool. Jahrb.* (*Phys.*) **60**, 1–12.

Haubenstock, H. (1961) Independence of sex ratio and diet induced changes in blood pH of mice. Cited by Weir 1962.

Hauenschild, C. (1953) Neue Versuche über phaenotypische Geschlechtsbestimmung bei Polychaeten, *Naturwiss.* **40**, 186–189.

Hauenschild, C. (1953) Die phaenotypische Geschlechtsbestimmung bei *Grubea clavata*, *Zool. Jahrb.* (*Phys.*) **64**, 14–54.

Hauenschild, C. (1959) Weitere Kreuzungsversuche zur Frage der Geschlechts bestimmung bei dem Polychaeten *Grubea clavata*, *Z. Naturforsch.* **14**b, 89–92.

Hayashi, T. (1963) Karyotypic analysis of 83 cases of Down's syndrome in Harris County, Texas. *Texas Rep. Biol. Med.* **21**, 28–36.

Hayes, W. (1952) Recombination in *Bacterium coli* K-12: unidirectional transfer of genetic material. *Nature* (*Lond.*) **169**, 118.

Hayes, W. (1953) The mechanism of genetic recombination in *Eschericia coli*, *Cold Spring Harbor Symp.* **18**, 75–93.

Hayes, W. (1960) The bacterial chromosome. *Microbial Genetics*, Ed. by W. Hayes. Cambridge.

Hayes, W. (1960) *Sexuality in Bacteria*. Ed. by C. R. Austin. Sex differentiation and development. p. 115–122.

Hayes, W. (1964) The genetics of bacteria and their viruses, Blackwell, Oxford.

Hauschteck, E. (1962) Die Cytologie der Pädogenese und der Geschlechtsbestimmung einer heterogonen Gallmücke, *Chromosoma* **13**, 163–182.

Heilbronn, A. (1948) Über die Genetik von Monoecie und Getrenntgeschlechtigkeit bei *Bryonia* Bastarden, *Proc. 8th Intern. Congr. Genet. Hereditas* (Suppl.) 590–591.

Heilbronn, A. (1953) Ueber die Rolle des Plasmas bei der Geschlechtsbestimmung der Bryonien, *Rev. fac. sc. Univ. Istanbul* (Sér. B) **18**, 205–207.

Heilbronn, A. and Basarman, M. (1942) Über die F_2 der *Bryonia* Bastarde und ihre Bedeutung für das Problem der Geschlechtsrealisation, *Rev. fac. sci. univ. Istanbul* (Ser. B) **7**, 139–144.

Heitz, E. (1928) Das Heterochromatin der Moose. *Jahrb. wiss. Bot.* **69**, 762–818.

Herbst, C. (1928) Untersuchungen zur Bestimmung des Geschlechts. I. Mitt.: Ein neuer Weg zur Lösung des Geschlechtsbestimmungsproblems bei *Bonellia viridis*, *Sitz.-Ber. Heidelberger Akad. d. Wiss.* **2**.

Herbst, C. (1929) Untersuchungen zur Bestimmung des Geschlechts. II. Weitere Experimente über die Vermännlichung indifferenter *Bonellia-Larven* durch künstliche Mittel, *Sitz.-Ber. d. Heidelberger Akad. d. Wiss.* **16**.

Herbst, C. (1932) Untersuchungen zur Bestimmung des Geschlechts. III. Die Vermännlichung der Larven von *Bonellia viridis* durch Kupferspuren, *Naturwiss.* **20**, 375.

Herbst, C. (1935) Untersuchungen zur Bestimmung des Geschlechts. IV. Die Abhängigkeit des Geschlechts vom Kaliumgehalt des umgehenden Mediums bei *Bonellia viridis*, *Arch. Entwicklungsmech.* **132**, 576–599.

Herbst, C. (1936a) Untersuchungen zur Bestimmung des Geschlechts. V. Die Notwendigkeit des Magnesiums für die Lebenserhaltung u. Weiterentwicklung der *Bonellia* Larven und seine Bedeutung für die geschlechtliche Differenzierung derselben, *Arch. Entwicklungsmech.* **134,** 313.

Herbst, C. (1936b) Untersuchungen zur Bestimmung des Geschlechts. VI. Neue Gedanken zur Geschlechtsbestimmung bei Tieren, *Arch. Entwicklungsmech.* **135,** 178.

Herbst, C. (1937) Untersuchungen zur Bestimmung des Geschlechts. VII. Ueber die Bedeutung des SO_4-Jons für die Weiterentwicklung u. geschlechliche Differenzierung der *Bonellia* Larven und über den Einfluss des Erhöten Ca Gehaltes im SO_4-armen Medium auf diese Prozesse, *Arch. Entwicklungsmech.* **136,** 147–168.

Herbst, C. (1939) Untersuchungen zur Bestimmung des Geschlechts. IX. Der Einfluss des Glycerins auf die Geschlechtsbestimmung bei *Bonellia*, *Arch. Entwicklungsmech.* **139,** 282.

Herbst, C. (1940) Untersuchungen zur Bestimmung des Geschlechts. X. Über *Bonellia* Weibchen mit Spältförmiger Leibeshöle und ihre Bedeutung für meine Hydratationstheorie, *Arch. Entwicklungsmech.* **140,** 252–284.

Herlant Meewis, H. (1958) La reproduction asexuée chez les Annelides, *Ann. Biol.* **34,** 133–166.

Hirschorn, K., Decker, W. M. and Cooper, H. L. (1960) True hermaphroditism with XY/XO mosaicism, *Lancet* ii, 319.

Hoffmann, A. (1956) Untersuchungen über die Geschlechtsdifferenzierung bei haploiden und diploiden Gametophyten von *Bryum capillare*, *Z. Vererbungslehre* **87**, 753–768.

Hoffmann, A. (1957) Untersuchungen über die Geschlechtsdifferenzierung bei haploiden und diploiden Gametophyten von *Barbula unguicolata*, *Z. Vererbungslehre*. **88**, 374–387.

Hofmeyr, J. D. J. (1938) Genetical studies of *Carica papaya* L. I-II, *Union S. African Dept. Agr. and Forestry Sci. Bull.* **187**, 5–64.

Hofmeyr, J. D. J. (1939) Some suggestions on the mechanism of sex determination in *Carica papaya*, *S. African J. Sci.* **36**, 288–290.

Howard, H. W. (1942) The genetics of *Armadillidium vulgare* Latr. II. Studies on the inheritance of monogeny and amphogeny, *J. Genet.* **44,** 143–159.

Howard, H. W. (1958) The genetics of *Armadillidium vulgare*. 4. Lines breeding true for amphogeny and theligeny, *Genetics*. **56,** 1–10.

Hughes Schrader, S. (1925) Cytology of hermaphroditism in *Icerya purchasi*, *Z. Zellforsch.* **2,** 264–292.

Hughes Schrader, S. (1927) Origin and differentiation of the male and female germ cells in the hermaphrodite of *Icerya purchasi*, *Z. Zellforsch.* **6,** 509–540.

Hughes Schrader, S. (1935) The chromosome cycle of *Phenococcus*, *Biol. Bull.* **69,** 462–468.

Hughes Schrader, S. (1942) The chromosomes of *Nantococcus schraderae* and the meiotic division figures of male Llaveinae coccids, *J. Morph.* **70,** 261–299.

Hughes Schrader, S. (1948) Cytology of Coccids, *Adv. Genet.* **2,** 127–203.

Hughes Schrader, S. (1950) The chromosomes of mantids in relation to taxonomy, *Chromosoma* **4,** 1–55.

Humphrey, R. R. (1938) Sex differentiation in gonads developed from transplants of the intermediate mesoderm of *Amblystoma*, *Biol. Bull.* **55,** 317.

HUMPHREY, R. R. (1945) Sex determination in amblystomid salamanders: a study of the progeny of females experimentally converted into males, *Am. J. Anat.* **76,** 33.

HUMPHREY, R. R. (1948) Reversal of sex in females of genotype WW in the axolotl and its bearing upon the role of the Z chromosomes in the development of the testis, *J. Exp. Zool.* **109,** 171–185.

HUTT, F. B., RICKARD, C. G. and FIELD, R. A. (1948) Sex-linked haemophilia in dogs, *J. Hered.* **39,** 3–9.

JACOB, F., SCHAEFFER, P. and WOLLMANN, E. L. (1960) Episomic elements in Bacteria. *Microbial Genetics,* Cambridge.

JACOB, F. and WOLLMANN, L. (1958) Les épisomes, éléments génétiques ajoutés, *C. R. Acad. Sci.* **247,** 154–156.

JACOB, F. and WOLLMANN, E. L. (1958) Genetic and physical determination of chromosomal segments in *E. coli.* The biological replication of macromolecules. *Symp. Exp. Biol.* **12,** 75–92.

JACOBS, P. A. *et al.* (1960) Abnormalities involving the X chromosome in women, *Lancet* **i,** 1213–1216.

JACOBS, P. A. *et al.* (1961) Cytogenetic studies in primary amenorrhaea, *Lancet* i, 1183.

JANICK, J. and STEVENSON, E. C. (1955) Genetics of the monoecious character in spinach, *Genetics* **40,** 429–437.

JANICK, J. and STEVENSON, E. C. (1955) Environmental influences on sex expressions in monoecious lines of spinach, *Proc. Amer. Soc. Horticult. Sci.* **65,** 416–422.

JOHNSON, G. (1961) Contribution a l'étude de la détermination du sexe chez les Oniscoides, *Bull. Biol. Fr. et Belg.* **95,** 117–267.

JONES, D. F. (1934) Unisexual Maize plants and their bearing on sex differentiation in other plants and in animals, *J. Genet.* **19,** 552–567.

KALLMANN, K. D. and HARRINGTON, R. W. (1964) Evidence for the existence of homozygous clones in the self-fertilizing hermaphroditic Teleost *Rivulus marmoratus, Biol. Bull.* **126,** 101–114.

KALMUS, H. and SMITH, C. A. B. (1960) Evolutionary origin of sexual differentiation and the sex ratio, *Nature* (*Lond.*) **186,** 1004–1006.

KATAKURA, Y. (1959) Masculinization through implanting testes into the female *Armadillidium vulgare, Proc. Japan. Acad.* **35,** 95–98.

KATAKURA, Y. (1961) Hormonal control of development of sexual characters in the isopod Crustacea *Armadillidium vulgare, Annot. Zool. Japon* **34,** 60–71.

KAWAMURA, T. (1949) Studies on hybridization in Amphibians. II, *J. Sci. Hiroshima Univ.* (Ser. B) **11,** 61–70.

KENK, R. (1941) Induction of sexuality in the asexual form of *Dugesia tigrina, J. Exper. Zool.* **85,** 55.

KERR, W. E. and LAIDLAW, H. H. (1956) General genetics of bees, *Adv. Genet.* **8,** 109–153.

KIHARA, H. (1930) Karyologische Studien an *Fragaria* mit besonderer Berücksichtigung der Geschlechtschromosomen, *Cytologia* **1,** 345–357.

KIHARA, H. (1953) Genetics of *Bombyx* and *Drosophila:* a comparison of materials, methods and results, *Seiken Zihō, Rep. Kihara Inst. Biol. Res.* **6,** 15–29.

KINNE, O. (1952) Zur Biologie u. Physiologie von *Gammarus duebeni.* III. Zahlenverhältnisse d. Geschlechter u. Geschlechtsbestimmung, *Kieler Meeresforsch.* **9,** 126–133.

KINNE, O. (1953) Zur Biologie u. Physiologie von *Gammarus duebeni.* VII. Über die temperatur Abhängigkeit der Geschlechtsbestimmung, *Biol. Zbl.* **72,** 260–270.

KNAPP, E. (1936) Heteroploidie bei *Sphaerocarpus, Ber. deutsch. bot. Ges.* **54,** 346–361.

KNAPP, E. and HOFFMANN, I. (1939) Geschlechtsumwandlung bei *Sphaerocarpus* durch Verlust eines Stückes des X chromosomes, *Chromosoma* **1,** 130–146.

KNIEP, H. (1928) *Die Sexualität der niederen Pflanzen.* Fischer, Jena.

KÖHLER, D. (1964) Geschlechtsbestimmung bei Blütenpflanzen, *Ergebn. Biol.* **27,** 98–115.

KORSCHELT, E. (1927) *Regeneration und Transplantation. I. Regeneration.* Berlin.

KORSCHELT, E. (1942) Weiteres über die Dauer der ungeschlechtlichen Fortpflanzung des *Ctenodrilus monostylos*, *Zool. Anz.* **137**.

KOSSWIG, C. (1930) Die Geschlechtsbestimmung bei den Bastarden von *Xiphophorus helleri* und *Platypoecilus maculatus* und deren Nachkommen, *Z. ind. Abstamm. u. Vererbungsl.* **54**, 263–267.

KOSSWIG, C. (1933a) Die Geschlechtsbestimmungsanalyse bei Zahnkarpfen. *Z. ind. Abst. u. Vererbungsl.* **67**, 200–205.

KOSSWIG, C. (1933b) Genotypische und phänotypische Geschlechtsbestimmung bei Zähnkarpfen. III. Farbfaktoren als relative Geschlechtsrealisatoren, *Arch. Entwicklungsmech.* **128**, 394–446.

KOSSWIG, C. (1935a) Genotypische und phänotypische Geschlechtsbestimmung. IV. Über polyfaktorielle Geschlechtsbestimmung, *Arch. Entwicklungsmech.* **133**, 140–155.

KOSSWIG, C. (1935b) Idiotypus und Geschlecht, *Z. ind. Abst. u. Vererbungsl.* **70**, 377–401.

KOSSWIG, C. (1939a) Die Geschlechtsbestimmung in Kreuzungen zwischen *Xiphophorus* und *Platypoecilus*, *C.R. Fac. Sci. Univ. Istanbul* **4**, 1–54.

KOSSWIG, C. (1939b) Die Geschlechtsbestimmungs Analyse bei Zahnkarpfen, *Rev. Fac. Sci. Univ. Istanbul* **4**, 1–32.

KOSSWIG, C. (1954) Zum Geschlechtsbestimmungsproblem bei den Zahnkarpfen, *Rev. Fac. Sci. Univ. Istanbul* **19**, 187.

KOSSWIG, C. (1959) Beiträge zur genetischen Analyse xiphophoriner Zahnkarpfen, *Biol. Zbl.* **78**, 711–718.

KOSSWIG, C. and ÖKTAY, M. (1955) Die Geschlechtsbestimmung bei den Xiphophorini, *Publ. Hydrob. Res. Inst. Istanbul*, B, **2**, 133–156.

KOSSWIG, C. (1964) Polygenic sex determination, *Experientia*, **20**, 1–10.

KUHN, E. (1939) Selbstbestäubungen subdiöcischer Blütenpflanzen, ein neuer Beweis für die genetische Theorie der Geschlechtsbestimmung, *Planta* **30**, 457–470.

KUSCHAKEWITSCH, S. (1923) Die Entwicklungsgeschichte der Keimdrüsen von *Rana esculenta*, *Festchr.* 60 *Geb. R. Hertwig* **2**, 63–225.

LA GRECA, M. and BACCI, G. (1962) Una nuova specie di *Ophryotrocha* delle coste tirreniche, *Boll. Zool.* **29**, 13–23.

LAMPEL, G. (1958) Die symbiontischen Einrichtungen im Rahmen des Generationswechsels monözischer und heterözischer Pemphiginen der Schwartz-und Pyramidenpappel, *Z. Morph. u. Ökol. Tiere* **47**, 403–435.

LAMPEL, G. (1959) Geschlecht und Symbiose bei den Pemphiginen, *Z. Morph. u. Ökol. Tiere* **48**, 320–348.

LAMS, H. (1908) Les divisions des spermatocytes chez la fourmi (*Camponotus herculeanus*), *Arch. Zelleforsch.* **1**, 28–37.

LAUTERBORN, R. (1898) Über die cyklische Fortpflanzung limnetischer Rotatorien, *Biol. Zbl.* **18**.

LEBEDEFF, C. A. (1934) A gene for intersexuality in *Drosophila virilis*, *Amer. Nat.* **68**, 68–69.

LEBEDEFF, C. A. (1939) A study of intersexuality in *Drosophila virilis*, *Genetics* **24**, 553–586.

LEDERBERG, J. (1955) Recombination mechanisms in bacteria, *J. Cell. Comp. Phys.* **45**, (Suppl.), 75–107.

LEDERBERG, J. and TATUM, E. L. (1946) Novel genotypes in mixed cultures of biochemical mutants of bacteria, *Cold Spring Harb. Symp.* **11**, 113–114.

LEGRAND, J. J. (1947) Cas d'inversion du sexe chez *Porcellio dilatatus*, *C. R. Acad. Sci.* **224**, 1029–1031.

LEGRAND, J. J. (1952) Contribution à l'étude expérimentale et statistique de la biologie d'*Anilocra physodes*, *Arch. Zool. exper. génér.* **89**, 1–55.

LEGRAND, J. J. (1956) Détermination polyfactorielle du sexe et double déterminisme des caractères sexuels mâles chez les Oniscoides. *C. R. Acad. Sci.* **243**, 1565–1568.

LEGRAND, J. J. (1958) Mise en évidence histologique et expérimentale d'un tissue androgène chez les Oniscoides, *C. R. Acad. Sci.* **247**, 1238–1241.

LEGRAND, J. J. and LEGRAND, H. (1947) L'intersexualité chez l'Oniscoide *Porcellio dilatatus*, *C. R. Acad. Sci.* **224**, 853.

LEGRAND, J. J. and JUCHAULT, P. (1960) Disposition metamerique du tissue sécréteur de l'hormone mâle chez les differents types d'Oniscoides, *C. R. Acad. Sci.* **250**, 764–766.

LEGRAND, J. J. and JUCHAULT, P. (1960a) Structure et origine de la glande androgène chez *Helleria brevicornis* (Oniscoidea, Tylidae). *C. R. Soc. Biol.* **154**, 676–678.

LEJEUNE, J., TURPIN, R. and GAUTHIER, M. (1959) Le mongolisme, premier exemple d'aberration autosomique humaine, *Ann. Génét.* **1**, 41–49.

LERCHE, W. (1937) Untersuchungen über Entwicklung und Fortpflanzung in der Gattung *Dunaliella*, *Arch. Protistenk.* **88**, 236–268.

LEWIN, R. A. (1954) Sex in unicellular algae. *Sex in Microorganisms*, Amer. Assoc. Adv. Sci. Wash. p. 100–133.

LINDAHL, P. E. (1958) Separation of bull spermatozoa carrying X and Y chromosomes by counter streaming centrifugation, *Nature* (*Lond.*) **181**, 784.

LINDAHL, P. E. and SUNDALL, G. (1958) Sex ratio in the golden hamster before uterine implantation, *Nature* (*Lond.*) **182**, 1392.

LINDSTEN, J. (1961) New type of chromosomal mosaicism in ovarian disgenesis, Lancet II, 1228.

LINDSTEN, J. (1963) *The Nature and Origin of X Chromosome Aberrations in Turner's Syndrome*. Stockholm, Almquist & Wiksell.

LOOMIS, W. F. (1957) Sexual differentiation in *Hydra*, *Science* **126**, 735–739.

LOOSANOFF, V. (1939) Development of the primary gonad and sexual phases in *Venus mercenaria*, *Biol. Bull.* **72**, 389–405.

LORBEER, G. (1927) Untersuchungen über Reduktionsteilung und Geschlechtsbestimmung bei Lebermoosen, *Z. ind. Abst. u. Vererbungsl.* **44**, 1–109.

LORBEER, G. (1934) Die Zytologie der Lebermoose mit besonderer Berücksichtigung allgemeiner Chromosomenfragen, *Jb. wiss. Bot.* **80**, 567–817.

LORBEER, G. (1936) Die Umwandlung eines haploiden, genotypisch weiblichen Gametophyten von *Sphaerocarpus Donnellii* in einen männlichen mit Hilfe von Röntgenstrahlen, *Planta* **25**, 70–83.

LORBEER, G. (1938) Über das Vorkommen von drei verschiedenen Geschlechtsrealisatoren bei den Lebermoosen, *Planta* **27**, 708–717.

LUCAS, A. and FRANC, A. (1962) Sur les modalités de l'hermaphroditisme successif chez *Chlamys varia*, *C. R. Acad. Sci.* **254**.

LUCAS, C. E. (1947) The ecological effects of external metabolites. *Biol. Rev.* **22**, 270–295.

LUNTZ, A. (1926) Untersuchungen über den Generationswechsel der Rotatoria. I. Die Bedingungen des Generationswechsel. *Biol. Zbl.* **46**, 233–256, 257–278.

LUNTZ, A. (1929) Untersuchungen über den Generationswechsel der Rädertiere. II. Der Zychlische Generationswechsel von *Brachionus bakeri*, *Biol. Zbl.* **49**, 193–211.

LYON, M. F. (1961) Gene action in the X chromosome of the mouse. *Nature* (*Lond.*) **190**, 372–373.

LYON, M. F. (1962) Sex chromatin and gene action in the mammalian X chromosome, *Amer. J. Hum. Genet.* **14**, 135–148.

LYON, M. F. (1963) Attempts to test the inactive X theory of dosage compensation in mammals, *Genet. Research* **4**, 93–103.

McCLUNG, C. E. (1902) The accessory chromosome sex determinant? *Biol. Bull.* **3**, 43.

MACKENSEN, O. (1950) Viability and sex determination in the honey bee (*Apis mellifera* L.), *Genetics* **36**, 500–509.

McKEOWN, T. and LOWE, C. R. (1951) The sex ratio of stillbirths related to cause and duration of the pregnation, *Human Biol.* **23**, 41–60.

MACINTYRE, P. (1961) Spontaneous sex reversal of genotypic males in the platyfish, *Genetics*, **46**, 575–580.

MAGNI, G. E. (1952) Sex ratio in *Drosophila bifasciata*, *Ist Lombardo Sci. Lett.* **85**, 391–411.

MAGNI, G. E. (1954) Thermic cure of cytoplasmic sex ratio in *Drosophila bifasciata*, *Caryologia* **6**, 1213–1216.

MAINX, F. (1933) *Die Sexualität als Problem der Genetik*. Fischer, Jena.

MAKINO, S. and SASAKI, M. (1960) A study of the somatic chromosomes of the Japanese, *Japan J. Genet.* **35**, 228–237.

MALOGOLOWKIN, C. (1958) Maternally inherited "sex ratio" conditions in *Drosophila willistoni* and *Drosophila paulistorum*, *Genetics* **43**, 274–286.

MALOGOLOWKIN, C. and POULSON, D. F. (1957) Infective transfer of maternally inherited abnormal sex ratio in *Drosophila willistoni*, *Science* **126**, 32.

MALOGOLOWKIN, C., POULSON, D. F. and WRIGHT, E. Y. (1959) Experimental transfer of maternally inherited abnormal sex ratio in *Drosophila willistoni*, *Genetics* **44**, 59–74.

MANNING, F. J. (1949) Sex determination in the honey bee, *Microscope* **7**, 175–180.

MANNING, F. J. (1950) Sex determination in the honey bee. VI. The number and size of the chromosomes in the honey bee, *Microscope* **7**, 303–305.

MARCHAL, E. and E. (1906) Recherches experimentales sur la sexualité des spores chez les mousses dioiques, *Mém. Acad. Sci. Belg.* (2me Sér.) **1**, 50.

MARCHAL, E. and E. (1907) Aposporie et sexualité chez les mousses. I, *Bull. Acad. Roy. Belg.* (Cl. Sci.), 765–789.

MARCHAL, E. and E. (1909) Aposporie et sexualité chez les mousses. II, *Bull. Acad. Roy. Belg.* (Cl. Sci.), 1249–1288.

ARCHAL, P. (1913) Contribution a l'étude de la biologie des Chermes, *Ann. Sci. Nat. Zool.*
M **18**, 153–385.

MATTHEY, R. (1949) *Les Chromosomes des Vertébrés.* Lausanne, Rouge.

MAUPAS, E. (1891) Determination de la sexualité chez *Hydatina*, *C. R. Acad. Sci.* **113**, 388–390.

MAUPAS, E. (1919) Experiences sur la reproduction asexuée des Oligochetes, *Bull. Biol. Belgique*. **53**.

MEHLING, E. (1915) Ueber die gynandromorphen Bienen des Eugsterchen Stockes, *Verh. phys. med. Ges. Würzburg. N.F.* **43**.

METZ, C. W. (1938) Chromosome behaviour, inheritance and sex determination in *Sciara*, *Amer. Natur.* **72**, 485–520.

METZLER, S. (1955) Über die Beeinflussbarkeit des Geschlechtsverhältnisses bei *Cyclops viridis*, *Naturwiss.* **42**, 517–518.

METZLER, S. (1957) Über die Beeinflusssbarkeit des Geschlechtsverhältnisses von *Cyclops viridis* durch Aussenfaktoren, *Zool. Jahrb.* (*Phys.*) **67**, 81–110.

MEVES, F. (1904) Über "Richtungskörperbildung" im Hoden von Hymenopteren, *Anat. Anz.* **24**.

MEVES, F. (1907) Die Spermatozytenteilungen bei der Honigbiene, *Ark. mikr. Anat.* **70**, 414–491.

MITTWOCH, U. (1964) Sex chromosomes and sex chromatin, *Nature* **204**, 1032–1034.

MOEWUS, F. (1935) Über die Vererbung des Geschlechts bei *Polytoma pascheri* und bei *Polytoma uvella*, *Z. Vererbungsl.* **69**.

MOEWUS, F. (1936) Faktorenaustausch, insbesondere der Realisatoren bei *Chlamydomonas* Kreuzungen, *Ber. Deutsch. Bot. Ges.* **54**, 48–57.

MOEWUS, F. (1937) Methodik und Nachträge zu den Kreuzungen zwischen *Polytoma* Arten und zwischen *Protosiphon* Rassen, *Z. Vererbungsl.* **73**, 63–107.

MONROY, A. (1939) Sulla localizzazione delle cellule genitali primordiali in fasi precoci di sviluppo, *Arch. Ital. Anat. Embr.* **41**, 368–389.

MONTALENTI, G. (1941) Studi sull'ermafroditismo dei Cimotoidi. I. *Emetha audouini* e *Anilocra physodes*, *Pubbl. Staz. Zool. Napoli* **18**, 337–394.

MONTALENTI, G. (1950) Evoluzione della sessualità. I problemi biologici della sessualità, *Acc. Naz. Lincei Quad.* **22**, 80–102.

MONTALENTI, G. (1960) Alcune considerazioni sull'evoluzione della determinazione del sesso, *Acc. Naz. Lincei. Quad.* **47**, 153–181.

MONTALENTI, G. and BACCI, G. (1951) Osservazioni e ipotesi sulla determinazione del sesso negli ermafroditi, *Scientia Genetica* **4**, 5–12.

MONTALENTI, G. and VITAGLIANO TADINI, G. (1963). Variability of sex ratio and sex determining mechanism in *Asellus*. *Genetics Today* **1**, 186–187.

MOORE, K. L. and BARR, M. L. (1954) Nuclear morphology according to sex in human tissues, *Acta Anat.* **21**, 197.

MORESCALCHI, A. (1964) Il corredo cromosomico di *Discoglossus pictus:* cromosomi sessuali, spiralizzazione cromosomica e zone eterochromatiche. *Caryologia* **17**, 327–345.

MORGAN, T. H. (1906) The male and female eggs of the phylloxerans of the hickories, *Biol. Bull.* **10**, 201–206.

MORGAN, T. H. (1909) A biological and cytological study of sex determination in Phylloxerans and Aphids, *J. Exp. Zool.* **7**, 239–351.

MORGAN, T. H. (1912) The elimination of the sex chromosomes from the male producing eggs of Phylloxerans, *J. Exp. Zool.* **12**, 379–398.

MORGAN, T. H. (1915) The praedetermination of sex in Phylloxerans and Aphids, *J. Exp. Zool.* **19**, 285–321.

MORGAN, T. H. and BRIDGES, C. B. (1919) The origin of gynandromorphs, *Carn. Inst. Publ.* **278**, 1.

MORGAN, T. H., BRIDGES, C. B. and STURTEVANT, H. H. (1925) The genetics of *Drosophila*, *Bibl. Gen.* **2**, 1.

MORTIMER, C. H. (1936) Experimentelle u. cytologische Untersuchungen über den Generationswechsel der Cladoceren, *Zool. Jahrb.* (*Phys.*) **56**, 323–388.

MULDAL, S. (1952) The chromosomes of the earthworms. I. The evolution of polyploidy, *Heredity* **6**, 55–76.

MÜLLER, H. (1952) Über die Sexualität des Polychaeten *Ophryotrocha puerilis*, ihre determination und ihren Einfluss auf Drüsentätigkeit, *Z. Morph. Ökol. Tiere* **52**, 1–32.

MÜLLER, H. J. (1932) Some genetic aspects of sex, *Amer. Nat.* **66**, 118–138.

MÜLSOW, K. (1911) Chromosomenverhältnisse bei *Ancyracanthus cystidicola*, *Zool. Anz.* **38**, 484–486.

MURRAY, M. J. (1940) The genetics of sex determination in the family Amaranthaceae, *Genetics* **25**, 409–431.

MUSTARD, J. F., ROSWELL, H. C., ROBINSON, G. A., HOEKSEMA, T. D., DOWNIE, H. G. (1960) Canine hemophilia B. *Brit. J. Haemat.* **6**, 259–266.

NACHTSHEIM, H. (1913) Cytologische Studien über die Geschlechtsbestimmung bei der Honigbiene (*Apis mellifica*), *Arch. f. Zellforsch.* **11**, 196–241.

NACHTSHEIM, H. (1920) Zytologische u. experimentelle Untersuchungen über die Geschlechtsbestimmung bei *Dinophilus apatris*, *Ark. mikr. Anat.* **93**, 17–140.

NANNEY, D. L. (1953) Mating type determination in *Paramecium aurelia*, a model of nucleo cytoplasmic interaction. *Proc. Nat. Acad. Sci.* **39**, 113–119.

NANNEY, D. L. (1957) Mating type inheritance at conjugation in variety 4 of *Paramecium aurelia*, *J. Protozool.* **4**, 89–95.

NANNEY, D. L. (1960) The relationship between the mating type and the H serotype systems in *Tetrahymena*, *Genetics* **45**, 1351–1358.

NARBEL-HOFSTETTER, M. (1946) La cytologie de la parthénogenèse chez *Apterona helix*, *Rev. Suisse Zool.* **53**, 625–681.

NARBEL-HOFSTETTER, M. (1963) Les altérations de la méiose chez les animaux parthénogénétiques, *Protoplasmatologia* **6**, 160 pp.

NEGODI, G. (1929) Ricerche sulla distribuzione e trasmissione dei sessi in *Urtica caudata*, *N. Giorn. Bot. Ital.* **36**, 60.

NELSON REES, W. A. (1960) A study of sex predetermination in the Mealy Bug *Planococcus citri*, *J. Exp. Zool.* **144**, 111–137.

NELSON REES, W. A. (1962) The effect of radiation damaged heterochromatic chromosomes in the Mealy Bug *Planococcus citri*, *Genetics* **47**, 661–684.

NIEUWKOOP, P. D. (1950) Causal analysis of the early development of the primordial germ cells and the germ ridges in Urodeles. *Arch. Anat. micr. Morph. exp.* **39**, 258–268.

NIGON, V. and ROMAN, E. (1952) Le déterminisme du sexe et le development cyclique de *Strongyloides ratti*, *Bull. Biol. Fr. Belgique.* **85**, 187–225.

NIIYAMA, H. (1950) The XY mechanism of the sex chromosome in the male of *Anisogammarus anandalei*, *Annot. zoo. Japan.* **23**, 58–62.

NIIYAMA, H. (1959) A comparative study of the chromosomes in Decapods, Isopods and Amphipods, with some remarks on cytotaxonomy and sex determination in the Crustacea, *Mem. Fac. Fish. Hokkaido Univ.* **7**, 1–60.

NIKOLEI, E. (1961) Vergleichende Untersuchungen zur Fortpflanzung heterogoner Gallmücken unter experimentellen Bedingungen, *Z. Morphol. Ökol. Tiere* **50**, 281–329.

NITSCH, J. P., KURTZ, E. B., LIVERMAN, J. L. and WENT, F. W. (1952) The development of sex expression in cucurbit flowers, *Amer. J. Bot.* **39,** 32–43.

NOWINSKY, W. W. (1934) Die vermännlichende Wirkung fraktionierter Darmextrakte des Weibchens auf die Larven der *Bonellia viridis*, *Pubbl. Staz. Zool. Napoli.* **14,** 110–145.

ODHNER, T. (1907) *Zur Anatomie der Didymozoen.* Zool. Stud. Uppsala.

OGUMA, K. (1934) Studies on the sauropsid chromosomes: II. The cytological evidence proving female heterogamety in the lizard, *Arch. Biol.* **45,** 27–46.

OHMORI, N. (1936) Studies on the tropical rat mite, *Zool. Mag. Formosa* **48,** 627–637.

OHNO, S. (1961) Sex chromosomes and microchromosomes of *Gallus domesticus*, *Chromosoma* **11,** 484–498.

OHNO, S., BEÇAK, W. and BEÇAK, M. L. (1964) X autosome ratio and the behaviour pattern of individual X-chromosomes in placental Mammals, *Chromosoma* **15,** 14–30.

OHNO, S. and CATTANACH, B. M. (1962) Cytological study of an X Autosome translocation in *Mus musculus*, *Cytogenetics* **1,** 129–140.

OHNO, S. and HAUSCHKA, T. S. (1960) Allocycly of the X chromosome in tumors and normal tissues, *Cancer Res.* **20,** 541–545.

OHNO, S. and MAKINO, S. (1961) The single X nature of sex chromatin in man, *Lancet* **i,** 78–79.

ÖKTAY, M. (1959) Über Ausnamemännchen bei *Platypoecilus maculatus* und eine neue Sippe mit XX-Männchen und XX Weibchen, *Rev. Fac. Sci. Istanbul* **24,** 75–91.

OKUGAWA, K. I. (1957) An experimental study of sexual induction in the asexual form of Japanese freshwater Planarian *Dugesia gonocephala*, *Bull. Kyoto Gakugei Univ.* **11** (B). 8.

OMODEO, P. (1952) Cariologia dei Lumbricidae, *Caryologia* **4,** 173–274.

ONO, T. (1935) Chromosomen u. Sexualität von *Rumex acetosa*, *Sci. Rep. Tohoku Imp. Univ. Sapporo.* **10,** 41–210.

ONO, T. (1939) Polyploidy and sex determination in *Melandrium.* I, *Botan. Mag.* (*Tokyo*) **53,** 549–556.

ONO, T. (1940) Polyploidy and sex determination in *Melandrium.* II, *Botan. Mag.* (*Tokyo*) **54,** 225–230.

ORTON, J. H. (1912) An account of the natural history of the slipper limpet (*Crepidula fornicata*) with some remarks on its occurrence in the oyster ground on the Essex coast, *J. Mar. Biol. Assoc.* **9,** 437–478.

ORTON, J. H. (1927a) Observations and experiments on sex change in the European oyster (*O. edulis*), *J. Mar. Biol. Assoc.* **14,** 967–1045.

ORTON, J. H. (1928) Observations on *Patella vulgata.* I. Sex phenomena, breeding and shell growth, *J. Mar. Biol. Assoc.* **15,** 851–874.

OVERZIER, C. (1958) Klinik der Störungen der embryonalen Geschlechtsdifferenzierung, *Verhandl. Deutsch. Ges. inn. Medizin* 1958, 425–479.

PADOA, E. (1936) Effetto paradossale (mascolinizzazione) sulla differenziazione sessuale dei girini di *Rana esculenta* trattati con ormone follicolare, *Mon. Zool. Ital.* **47,** 285–289.

PADOA, E. (1938) La differenziazione del sesso invertita mediante la somministrazione di ormoni sessuali. Ricerche con follicolina in *Rana esculenta*, *Arch. Ital. Anat. Embriol.* **40,** 122.

PADOA, E. (1942) Il differenziamento del sesso invertito mediante la somministrazione di ormoni sessuali e corticosurrenali, *Pubbl. Staz. Zool. Napoli* **19,** 185–223.

PADOA, E. (1946) Perché la partenogenesi facoltativa aploide determina sempre la mascolinità, *Mon. Zool. Ital.* **45,** 120–125.

PADOA, E. (1947) Differente sensibilità al testosterone dei genotipi maschili e femminili, *Arch. Zool. Ital.* **32,** 1–24.

PADOA, E. (1948a) Genotipi e fenotipi sessuali negli Anfibi, *Ric. Scient.* **18,** 3–17.

PADOA, E. (1948b) *Storia naturale del sesso.* Einaudi, Torino.

PADOA, E. (1950) Grenouilles et Sterols, *Arch. Anat. Microsc.* **39,** 314–336.

PADOA, E. (1959) L'origine dell'interrenale e della medulla dei corpi genitali nella *Rana esculenta*, *Monit. Zool. Ital.* **66,** 1–21.

Padoa, E. (1963) Le gonadi di girini di *Rana esculenta* da uova irradiate con ultravioletto, *Monit. Zool. Ital.* **71,** 238–249.

Panouse, J. B. (1943) Influence de l'ablation du penduncule oculaire sur la croissance de l'ovaire chez la crevette *Leander serratus, C. R. Acad. Sci.* **217,** 553.

Panouse, J. B. (1946) Recherches sur les phenomenes humoraux chez les Crustacés, *Ann. Inst. océan. Paris* (N.S.) **23,** 65–147.

Papazian, H. P. (1951) The incompatibility factors and a related gene in *Schizophyllum commune*, *Genetics* **36,** 441–459.

Parenti, U. (1960) Self-fertilization in *Ophryotrocha labronica*, *Experientia* **16,** 13–416.

Parenti, U. (1961) *Ophryotrocha puerilis siberti, O. hartmanni ed O. baccii* nelle acque di Roscoff, *Cah. Biol. Mar.* **2,** 437–445.

Parenti, U. (1962) Fecondazione e corredo cromosomico di *Paramermis contorta*, *Atti. Accad. Naz. Lincei* **32,** 699–702.

Parenti, U. (1962a) Ricerche sulla sessualità dei Mermetidi. 1. Rapporto sessi in una popolazione di *Paramermis contorta*, *Arch. Zool. Ital.* **47,** 209–224.

Parenti, U. (1962b) Ricerche sulla sessualità dei Mermetidi. 2. Infestazione sperimentale di *Paramermis contorta* in *Chironomus tentans*, *Boll. Zool.* **29,** 453–462.

Parenti, U. (1962c) Il differenziamento citosessuale in *Ophryotrocha hartmanni*. *Mon. Zool. Ital.* **69,** 106–119.

Parenti, U. (1965) Male and female influence of adult individuals on indifferentiated larvae of the parasitic Nematode *Paramermis contorta*. *Nature* (*Lond.*) **207,** 1105–1106.

Patane, L. (1955) Ricerche sui fenomeni della sessualità negli Isopodi terrestri, *Atti. Acc. Gioenia Sc. Nat. Catania*, Ser. VI, **10,** 44–54.

Pelseneer, P. (1895) L'hermaphroditisme chez les Mollusques, *Arch. de Biol.* **14,** 33–62.

Pelseneer, P. (1926) La proportion relative des sexes chez les Mollusques, *Mem. Acad. R. Bruxelles*, Ser. 8 no. 11, 1–258.

Pierantoni, U. (1913) Studio sullo sviluppo di *Icerya purchasi*. II. Origine ed evoluzione degli elementi sessuali maschili. Ermafroditismo, *Arch. Zool. Ital.* **7,** 27–49.

Pipkin, S. B. (1942) Intersex modifying genes in wild strains of *Drosophila melanogaster*, *Genetics* **27,** 286–298.

Pipkin, S. B. (1947) A search for sex genes in the second chromosome of *Drosophila melanogaster* using the triploid method, *Genetics* **32,** 592–607.

Pipkin, S. B. (1959) Sex balance in *Drosophila melanogaster*: aneuploidy of short regions of chromosome 3, using the triploid method, *Univ. Texas Publ.* 5914, 70–88.

Pipkin, S. B. (1960) Sex balance in *Drosophila melanogaster:* aneuploidy of long regions of chromosome 3, using the triploid method, *Genetics* **45,** 1205–1216.

Piquet, J. (1930) Détermination du sexe chez les Batraciens en function de la temperature, *Rev. Suisse. Zool.* **37,** 173–281.

Ponse, K. (1949) *La Différenciation du Sexe et l'Intersexualité chez les Vertébraés*. F. Rouge, Lausanne.

Pontecorvo, G. (1954) Mitotic recombination in the genetic system of filamentous fungi, *Caryologia* (Suppl.) **6,** 192–200.

Pontecorvo, G. and Roper, J. A. (1952) Genetic analysis without sexual reproduction by means of polyploidy in *Aspergillus nidulans*, *J. gen. Microbiol.* **6,** vii.

Pontecorvo, G., Roper, J. and Forbes, E. (1953) Genetic recombination without sexual reproduction in *Aspergillus niger*, *J. gen. Microbiol.* **8,** 198–210.

Poole, C. C. (1944) Genetics of cultivated cucurbits, *J. Hered.* **35,** 122–128.

Poulson, D. F. and Sakaguki, B. (1961) Nature of sex ratio agent in *Drosophila*, *Science* **133,** 1489–1490.

Pourriot, R. (1957) Influence de la nourriture sur l'apparition des femelles mictiques chez deux espèces et une variété de *Brachionus*, *Hydrobiol.* **9,** 60–65.

Powsner, E. R. and Berman, L. (1960) Number of chromosomes in the human cell, *Nature* **188,** 1045–1046.

Pringsheim, E. G. and Oudracek, K. (1939) Untersuchungen über die Geschlechtsvorgänge bei *Polytoma*, *Beih. zu Bot. Zbl.* Abt. A 59.

Punnett, R. C. (1933) Single or multiple sex factors? *Amer. Nat.* **67,** 473–476.

Ranzoli, F. (1954) L'inversione sessuale nel Polichete *Hydroides norvegica*, *Boll. Zool.* **21**, 125–128.

Ranzoli, F. (1959) Osservazioni preliminari nei primi stadi di sviluppo della gonade in Anfibi Urodeli. *Boll. Zool.* **27**, 445–455.

Ranzoli, F. (1962) Il falso gonocorismo nel ciclo biologico del Polichete *Hydroides norvegica*, *Arch. Zool. Ital.* **47**, 1–19.

Raper, J. R. (1947) On the distribution and sexuality of *Achlya ambisexualis*, *Am. J. Botany*. **34**, 31a.

Raper, J. R. (1953) Tetrapolar sexuality, *Quart. Rev. Biol.* **28**, 233–259.

Raper, J. R. (1954) Life cycles, sexuality, and sexual mechanisms in the Fungi. *Sex in Microorganisms*, Ed. by Wenrich pp. 43–81.

Reinhard, E. G. (1942) The reproductive role of the complementary males of *Peltogaster*, *J. Morph.* **70**, 389–402.

Reinhard, E. G. (1949) Experiments on the determination and differentiation of sex in the Bopyrid *Stegophrygus hyptius*, *Biol. Bull.* **96**, 17–31.

Reitberger, A. (1940) Die Cytologie des pädogenetischen Entwicklungscyclus der Gallmücke *Oligarces paradoxus*, *Chromosoma* **1**, 391–473.

Reverberi, G. (1944) La determinazione del sesso nei Crostacei e i fenomeni della castrazione parassitaria, *Rend. Ist. Lombardo Sci.* **78**, 217–246.

Reverberi, G. and Pitotti, M. (1942) Il ciclo biologico e la determinazione fenotipica del sesso di *Ione thoracica*, Bopiride parassita di *Callianassa laticauda*, *Pubbl. Staz. Zool. Napoli* **19**, 111–184.

Rick, C. M. and Hanna, G. C. (1943) Determination of sex in *Asparagus officinalis L.*, *Am. J. Bot.* **30**, 711–714.

Ris, H. and Kerr, W. E. (1952) Sex determination in the honey bee, *Evolution* **6**, 444–445.

Rothenbuhler, W. C. (1957) Diploid male tissue as new evidence on sex determination in honey bees, *Apis mellifera L.*, *J. Hered.* **48**, 160–168.

Rothenbuhler, W. C. (1958) Progress and problems in the analysis of gynandromorphic honey bees. *Proc. Tenth Intern. Congress Entom.* **2**, 867–874.

Rothenbuhler, W. C. (1958) Genetics and breeding of the honey bee, *Ann. Rev. Entomol.* **3**, 161–180.

Rothenbuhler, W. C., Gowen, J. W. and Park, O. W. (1952) Androgenesis with zygogenesis in gynandromorphic honey bees, *Science* **115**, 637–638.

Rothfels, K., Aspden, M. and Mollison, M. (1963) The W Chromosome of the budgerigar *Melopsittacus undulatus*, *Chromosoma* **14**, 459–467.

Rothschild (1960) X and Y spermatozoa, *Nature* (*Lond.*) **187**, 253–254.

Russell, L. B. (1961) Genetics of mammalian sex chromosomes, *Science* **133**, 1795–1803.

Russell, L. B. (1963) Evidence from six X chromosome translocations bearing on the single active X hypothesis. Geerts S. I. (ed.) *Genetics Today*. Oxford, Pergamon Press.

Rust, W. (1941) Genetische Untersuchungen über die Geschlechtsbestimmungstypen bei Zahnkarpfen unter besonderer Berücksichtigung von Artkreuzungen mit *Platypoecilus variatus*, *Z. ind. Abst. u. Vererbungsl.* **79**, 336–395.

Ruttner Kolisko, A. (1946) Über das Auftreten unbefruchteter "Dauereier" bei *Anurea aculeata* (*Keratella quadrata*), *Österr. Zool. Zeit.* **1**, 179–191.

Sabbadin, A. (1951) Studio morfologico e quantitativo sullo sviluppo delle gonadi di una razza differenziata di *Rana dalmatina*, *Arch. Zool. Ital.* **36**, 167–217.

Sager, R. and Granik, S. (1954) Nutritional control of sexuality in *Chlamydomonas*, *J. Gen. Physiol.* **37**, 729–742.

Saitoh, K. (1958) Studies of sexuality and of morphology in the gypsy moth *Lymantria dispar*. II. On the chromosomes of F_1 hybrids between the weak and strong races, *Jap. J. Gen.* **33**, 97–101.

Samuels, L. T. (1955) Metabolism of steroid hormones, *Progr. Chem. Fats & Lipids*. **3**, 395.

Sanderson, A. R. and Hall, D. W. (1951) Sex determination in the honey bee, *Evolution* **5**, 414–415.

Schleip, W. (1911) Das Verhalten des Chromatins bei *Angiostomum nigrovenosum*, *Arch. f. Zellforsch.* **7**, 87–138.

SCHMID, W. (1963) DNA replication patterns of human chromosomes, *Cytogenetics* **2,** 175–193.

SCHMIEDER, R. G. and WHITING, O. (1947) Reproductive economy in the Chalcidoid wasp *Melittobia*, *Genetics* **32,** 29–37.

SCHRADER, F. and HUGHES SCHRADER, S. (1926) Haploidy in *Icerya purchasi*, *Z. wiss. Zool.* **128,** 182–200.

SCOTT, A. C. (1936) Haploidy and aberrant spermatogenesis in a Coleopteran, *Micromalthus debilis*, *J. Morph.* **59,** 485–515.

SCOTT, A. C. (1941) Reversal of sex production in *Micromalthus*, *Biol. Bull.* **81,** 420–431.

SEILER, J. (1920) Geschlechtschromosomenuntersuchungen an Psychiden. I. Experimentelle Beeinflussung der Geschlechtsbestimmenden Reifeteilungen bei *Talaeporia tubulosa*, *Arch. Zellforsch.* **15,** 249–268.

SEILER, J. (1922) Geschlechtschromosomenuntersuchungen an Psychiden. IV. Die Parthenogenese der Psychiden, *Z. ind. Abst. u. Vererbungsl*, **31,** 1–99.

SEILER, J. (1946) Bemerkungen zu Goldschmidts Interpretation der intersexen Solenobien, *Arch. Julius Klaus Stift.* **21,** 273–275.

SEILER, J. (1947) Die Zytologie eines parthenogenetischen Rüsselkäfers *Othiorrhynchus sulcatus*, *Chromosoma* **3,** 88–109.

SEILER, J. (1949) Das Intersexualitätsphaenomenon, *Experientia* **5,** 425–438.

SHAW, R. F. (1958) The theoretical genetics of the sex ratio. *Genetics* **43,** 149–163.

SHETTLES, L. B. (1960) Nuclear morphology of human spermatozoa, *Nature* (*Lond.*) **186,** 648–649.

SHIFRISS, O. (1961) Sex control in cucumbers, *J. Hered.* **52.**

SHIFRISS, O. and GALUN, E. (1956) Sex expression in the cucumber, *Proc. Amer. Soc. Hort. Sci.* **67,** 479–486.

SHULL, A. F. (1929) Determination of types of individuals in Aphids, Rotifers and Cladocera, *Biol. Rev.* **4,** 218–248.

SMITH, G. W. (1913) Studies in the experimental analysis of sex. The effect of *Sacculina* on the storage of fat and glycogen, *Q. Journ. Micr. Sci.* **59,** 267–295.

SMITH, B. W. (1963) The mechanism of sex determination in *Rumex hastatulus*, *Genetics* **48,** 1265–1288.

SOKOLOW, I. (1934) Untersuchungen über die Spermatogenese bei den Arachniden, V. Über die Spermatogenese der Parasitidae, *Z. Zellforsch.* **21,** 42–109.

SONNEBORN, T. M. (1937) Sex, sex inheritance and sex determination in *Paramecium aurelia*, *Proc. Nat. Acad. Sci., Wash.* **23,** 378.

SONNEBORN, T. M. (1957) *Breeding systems, reproductive methods, and species problems in Protozoa.* The species problem, 155–324.

SOOST, R. K. (1951) Comparative cytology and genetics of asynaptic mutants in *Lycopersicum esculentum*, *Genetics* **36,** 410–434.

SORDI, M. (1962) Ermafroditismo proteroginico in *Labrus turdus* e in *L. merula*, *Monit. Zool. Ital.* **69,** 69–89.

SPURWAY, H. (1953) Genetics of specific and subspecific differences in European newts, *Symposia Soc. Exp. Biol.* **7,** 200–237.

SPURWAY, H. (1958) Hermaphroditism with self-fertilization, and the monthly extrusion of unfertilized eggs in the viviparous fish *Lebistes reticulatus*, *Nature* (*Lond.*) **180,** 1248–1251.

STEBBINS, G. L. (1950) *Variation and evolution in plants.* Columbia University Press, New York.

STEFANI, R. (1959) I fenomeni cariologici nella segmentazione dell'uovo ed i loro rapporti con la partenogenesi rudimentale ed accidentale negli Embiotteri, *Caryologia* **12,** 1–70.

STEFANI, R. (1960) I rapporti tra parassitosi, sterilità maschile e partenogenesi accidentale in popolazioni naturali di *Haploembia solieri* Ramb. anfigonica, *Riv. Parassit.* **21,** 277–287.

STEPHENS, G. J. (1952) Mechanisms regulating the reproductive cycle in the crayfish, *Cambarus*. I. The female cycle, *Physiol. Zool.* **25,** 70–84.

STERN, C. (1929) Untersuchungen über Aberrationen des Y-Chromosoms von *Drosophila melanogaster*, *Z. ind. Abst. u. Vererbungsl.* **51,** 253–353.

Stern, C. (1960) A mosaic of *Drosophila* consisting of 1X, 2X and 3X tissue and its probable origin by mitotic non-disjunction, *Nature* (*Lond.*) **186**, 179–180.

Stern, C. (1960) *Principles of Human Genetics*, 2nd ed. Freeman.

Stern, C. and Hadorn, E. (1938) The determination of sterility in *Drosophila* males without complete Y chromosome, *Amer. Natur.* **72**, 42–52.

Storch, O. (1924) Die Eizellen der heterogonen Rädertiere, *Zool. Jb.* (*Anat. Ontog.*) **45**, 309–401.

Storey, W. B. (1953) Genetics of the Papaya, *J. Hered.* **44**, 70–78.

Stunkard, H. W. (1959) Induced gametogenesis in a monogenetic Trematode: *Polystoma stellai*, *J. Parasit.* **45**, 389–394.

Sturtevant, A. H. (1920) Intersexes in *Drosophila simulans*, *Science* **51**, 325–327.

Sturtevant, A. H. (1921) Genetic studies in *Drosophila simulans*. III. Autosomal genes, *Genetics* **6**, 179–207.

Suomolainen, E. (1950) Parthenogenesis in animals, *Adv. Genet.* **3**, 193–253.

Takeda, N. (1950) Experimental studies on the effect of external agencies on the sexuality of a marine Copepod, *Phys. Zool.* **23**, 288–300.

Tanaka, Y. (1939) Non-disjunction of the sex chromosomes of the silkworm, *Japan J. Genet.* **15**, 359–361.

Tauson, A. (1927) Die Spermatogenese bei *Asplanchna intermedia*, *Z. Zellforsch.* **4**, 652–681.

Tazima, Y. (1943) Studies on chromosomal aberrations in the silkworm I (in Japanese), *Bull. Imp. Sericult. Exp. Stc. Tokyo* **11**, 525–604.

Teissier, G. (1928) Sur quelques disharmonies de croissance des Crustacés Brachyoure, *C. R. Soc. Biol.* **99**, 1934–1935.

Teissier, G. (1947) La relation d'allométrie. Sa signification statistique et biologique, *Biometrics* **4**, 14–53.

Thompson Bowen S. (1962) A gynandromorph of the brine shrimp, *Artemia salina*, *Genetics* **47**, 277–279.

Thompson Bowen, S. (1963) The genetics of *Artemia salina*. II. White eye, a sex-linked mutation, *Biol. Bull.* **124**, 17–23.

Timofeeff-Ressowsky, N. W. (1940) Zur Analyse des Polymorphismus bei *Adalia bipunctata*, *Biol. Zbl.* **60**, 130–137.

Tinturier Hamelin, E. (1959) La determination du sexe chez *Idothea baltica basteri*, *C. R. Acad. Sci.* **248**, 2660–2662.

Tinturier Hamelin, E. (1963) Polychromatisme et determination génétique du sexe chez l'éspèce polytypique *Idathea baltica*, *Cahiers Biol. Mar.* **4**, 473–591.

Tjio, J. H. and Levan, A. (1956) The chromosome number in man, *Hereditas* **42**, 1–6.

Torvik Greb, M. (1935) The chromosomes of *Habrobracon*, *Biol. Bull.* **68**, 25–34.

Trainor, F. R. (1958) Control of sexuality in *Chlamydomonas chlamydogama*, *Amer. J. Bot.* **45**, 621–626.

Traut, W. (1962) Zur Geschlechtsbestimmung bei *Gammarus duebueni* und verwandten Arten, *Z. Wiss. Zool.* **167**, 1–72.

Treillard, M. (1925) *Daphnia magna* en culture pure. Perennitè de la parthénogenèse: necessité de facteurs bactériennes pour l'apparition des formes sexuées, *C. R. Soc. Biol.* **93**.

Triantaphyllou, A. C. and Hirschmann, H. (1964) Reproduction in plant and soil Nematodes, *Ann. Rev. Phytopath.* **2**, 57–80.

Tucker, K. W. (1958) Automictic parthenogenesis in the honey bee. *Genetics* **43**, 299–316.

Uchida, T. (1937) Studies on the sexuality of Amphibia. III. Sex transformation in *Hynobius retardatus* by the function of high temperature, *J. Fac. Sci. Hokkaido Imp. Univ.* **6**, 59–70.

Ulrich, H. (1936) Experimentelle Untersuchungen über den Generationswechsel der heterogonen Cecydomyde *Oligarces paradoxus*, *Z. indukt. Abstamm. u. Vererb. Lehre* **71**, 1–60.

ULRICH, H. (1940) Über den Generationswechsel und seine Bedingungen, *Naturwiss.* **28,** 569–591.

ULRICH, H. (1962) Generationswechsel und Geschlechtsbestimmung einer Gallmücke mit viviparen Larven, *Verhandl. Deutsch. Zool. Gesell. Wien* 1962, 139–152.

VANDEL, A. (1931) *La parthénogenèse*, Doin, Paris.

VANDEL, A. (1938) Recherches sur la sexualité des Isopodes. III. Le déterminisme du sexe et de la monogenie chez *Trichoniscus provvisorius*, *Bull. Biol. Fr. et Belgique* **72,** 147–186.

VANNINI, E. (1941) La partecipazione del tessuto interrenale nell'organogenesi della gonade e nei processi di intersessualità giovanile della *Rana agilis*, *Rend. Accad. Ital.* **13,** 731–787.

VANNINI, E. (1950) Organogénèse des gonades et déterminisme du sexe chez les Amphibiens et les Amniotes, *Arch. Anat. Micr.* **39,** 295–313.

VANNINI, E. (1960) Risultati e programmi di ricerche sul problema del differenziamento citosessuale, *Rend. Accad. Sc. Ist. Bologna* (9) **7,** 1–16.

VANNINI, E. (1962) Esperimenti sul differenziamento regionale della gonade in embrioni e larve di *Bufo*, *Atti Accad. Sci. Bologna* **9,** 30–36.

VANNINI, E. and GHIRARDELLI, E. (1961) Sui fenomeni regolativi dell'abbozzo genitale in *Bufo bufo*, dopo asportazione del territorio presuntivo dell'organo di Bidder, *Rend. Accad. Naz. Lincei* **30,** 107–111.

VANNINI, E. and REGGIANI, M. P. (1959) Primi risultati di esperimenti di asportazione parziale dell'abbozzo genitale in embrioni di *Bufo bufo*, *Arch. Ital. Anat. Embr.* **64,** 301–317.

VANNINI, E. and SABBADIN, A. (1954) The relation of the inter-renal blastema to the origin of the somatic tissues of the gonad in frog tadpoles, *J. Embryol. Exp. Morph.* **2,** 275–289.

VEILLET, A. and GRAF, F. (1958) Dégénérescence de la glande androgène des Crustacés Decapodes parasités par les Rhizocéphales, *Bull. Soc. sci. Nancy* **18,** 123–127.

VITAGLIANO, TADINI G. (1958) Indagini sulle variazioni del rapporto sessi in *Asellus aquaticus*, *Atti Accad. naz. Lincei.* (Ser. 8) **24,** 335–341.

VORONTSOVA, M. A. and LIOSNER, L. D. (1960) *Asexual Propagation and Regeneration.* Pergamon Press, London.

WADDINGTON, C. H. (1956) Genetic assimilation of the *Bithorax* phenotype. *Evolution* **10,** 1–13.

WARMKE, H. E. (1946) Sex determination and sex balance in *Melandrium*, *Am. J. Bot.* **33,** 648–660.

WARMKE, H. E. and BLAKESLEE, A. F. (1939) Sex mechanism in polyploids of *Melandrium*, *Science*, 391–392.

WARMKE, H. E. and BLAKESLEE, A. F. (1939a) Effect of polyploidy upon the sex mechanism in dioecious plants, *Genetics* **24,** 88.

WARMKE, H. E. and BLAKESLEE, A. F. (1940) The establishment of a 4n dioecious race in *Melandrium*, *Am. J. Bot.* **27,** 751–762.

WEILER, C. and OHNO, S. (1962) Cytological confirmation of female heterogamety in the African water frog (*Xenopus laevis*), *Cytogenetics* **1,** 217–223.

WEIR, J. A. (1962) Hereditary and environmental influences on the sex ratio of PHH and PHL mice, *Genetics* **47,** 881–897.

WEIR, J. A. (1958) Sex ratio related to sperm source in mice, *J. Hered.* **49,** 223–227.

WEISMANN, A. (1876–1880) Beiträge zur Naturgeschichte der Daphnoiden, *I. Zeit. wiss. Zool.* **27, 28, 30, 33.**

WEISMANN, A. (1886a) *Die Bedeutung der sexuellen Fortpflanzung*. Jena, Fischer.

WEISMANN, A. (1886b) Richtungskörper bei parthenogenetischen Eier, *Zool. Anz.* **9.**

WELSHONS, W. J. and RUSSELL, L. B. (1959) The Y-chromosome as the bearer of male determining factors in the mouse, *Proc. Nat. Acad. Sci. U.S.* **45,** 560–566.

WESEMBERG LUND, C. (1930) Contributions to the biology of the Rotifera. Part II. *Kong. Danske Vidensk. Selsk. Sckrift. Naturvid. Math. Afd.* 9 *Rackke* II. **1,** 1–230, 15 Tav.

WESTERGAARD, M. (1940) Studies on cytology and sex determination in polyploid forms of *Melandrium album*, *Dansk. Bot. Ark.* **10,** 1–131.

WESTERGAARD, M. (1948) The relation between chromosome constitution and sex in the offspring of triploid *Melandrium*, *Hereditas* **34,** 257–279.

WESTERGAARD, M. (1953) Über den Mechanismus der Geschlechtsbestimmung bei *Melandrium album*, *Naturwiss.* **40,** 253–260.

WESTERGAARD, M. (1959) The mechanism of sex determination in dioecious flowering plants, *Adv. in. Gen.* **9,** 217–281.

WETTSTEIN, F. VON (1924a) Morphologie und Physiologie des Formwechsels der Moose auf genetischer Grundlage, *Z. ind. Abst. u. Vererbungsl.* **33,** 1–236.

WETTSTEIN, F. VON (1924b) Über Fragen der Geschlechtsbestimmung bei Pflanzen, *Naturwiss.* **12,** 761.

WHITE, M. D. J. (1946) The cytology of the Cecidomydae. II, *J. Morph.* **79,** 323–370.

WHITE, M. D. J. (1950) *Cytological Studies on Gall Midges.* University of Texas Publication **5007,** 1–78.

WHITE, M. D. J. (1954) *Animal Cytology and Evolution.* 2nd edn. Cambridge University Press.

WHITEHOUSE, H. L. K. (1949) Multiple allelomorphs heterothallism in the Fungi, *New Phytol.* **48,** 212–244.

WHITING, P. W. (1918) Sex determination and biology of a parasitic wasp, *Habrobracon brevicornis*, *Biol. Bull.* **34,** 250–256.

WHITING, P. W. (1928a) The relation between gynandromorphism and mutation in *Habrobracon*, *Amer. Nat.* **62,** 59–62.

WHITING, P. W. (1928b) Mosaicism and mutation in *Habrobracon*, *Biol. Bull.* **54,** 289–306.

WHITING, P. W. (1940) Multiple alleles in sex determination of *Habrobracon*, *J. Morph.* **66,** 323–355.

WHITING, P. W. (1943) Multiple alleles in complementary sex determination of *Habrobracon*, *Genetics* **28,** 365–382.

WHITING, P. W. (1954) The evolution of male haploidy, *Quart. Rev. Biol.* **20,** 231–260.

WHITNEY, D. D. (1912) Strains in *Hydatina senta*, *Biol. Bull.* **22,** 205–219.

WHITNEY, D. D. (1912) Reinvigoration produced by cross fertilization in *Hydatina senta*, *J. Exper. Zool.* **12,** 337–362.

WHITNEY, D. D. (1913) An explanation of the nonproduction of fertilized eggs by adult male-producing females in a species of *Asplanchna*, *Biol. Bull.* **25,** 318–321.

WHITNEY, D. D. (1929) The chromosome cycle in the Rotifer. *Asplanchna amphora*, *J. Morph.* **47,** 415–433.

WICKBOM, T. (1945) Cytological studies on Dipnoi, Urodela, Anura and Emys, *Hereditas* **31,** 241–346.

WIESE, L. (1960) Die diplogenotypische Geschlechtsbestimmung, *Fortschr. d. Zool.* **12,** 295–335.

WEISENSEE, N. (1916) Die Geschlechtsverhältnisse u. der Geschlechtsapparat bei *Anodonta*, *Z. wiss. Zool.* **115,** 262–335.

WILCCYNSKI, J. Z. (1959) Sex behaviour and sex determination in *Crepidula fornicata* L., *J. exp. Biol.* **36,** 34–39.

WILLIER, B. H., GALLAGHER, T. F. and KOCK, F. C. (1935) Sex modification in the chick embryo resulting from injections of male and female hormones, *Proc. Nat. Acad. Sci.* **21,** 625.

WILSON, E. (1905) The chromosomes in relation to the determination of sex in Insects, *Science* **22,** 564.

WILSON, E. (1906) Studies on chromosomes. III. The sexual differences of the chromosome groups in Hemiptera, with some considerations on the determination and inheritance of sex, *J. Exp. Zool.* **3,** 1–40.

WILSON, E. (1909) Studies on chromosomes. IV. The "accessory" chromosome in *Syromastes* and *Pyrrhocoris*, with a comparative review of the types of sexual difference of the chromosome groups, *J. Exp. Zool.* **6,** 69–99.

WILSON, E. (1912) Studies on chromosomes. VII. A review of the chromosome of *Nezara*, with some more general consideration, *J. Morph.* **22,** 71–110.

WILSON, E. (1928) *The Cell in Development and Heredity.* 3rd edn. MacMillan, New York.

WINGE, Ö. (1922) One sided masculine and sex-linked inheritance in *Lebistes reticulatus*, *J. Gen.* **12,** 145; *idem* (1922) *C. R. Labor. Carlsberg.* **14,** 1–20.

WINGE, Ö. (1923a) Crossing-over between the X and Y chromosomes in *Lebistes*, *J. Genet.* **13,** 201–217; *idem C. R. Labor. Carlsberg* (*Phys.*) **14,** 1–19.

WINGE, Ö. (1923b) On sex chromosomes, sex determination and preponderance of females in some dioecious plants, *C. R. Labor. Carlsberg* **15,** 1–26.

WINGE, Ö. (1930) On the occurrence of XX males in *Lebistes* with some remarks on Aida's so called non-disjunctional males in *Aplocheilus*, *J. Genet.* **23,** 69–76.

WINGE, Ö. (1931) X and Y-linked inheritance in *Melandrium*, *Hereditas* **15,** 127–165.

WINGE, Ö. (1932) The nature of sex chromosome, *Proc. Sixth Congr. Genetics* **1,** 343–355.

WINGE, Ö. (1934) The experimental alteration of sex chromosomes into autosomes and vice versa, as illustrated by *Lebistes*, *C. R. Labor. Carlsberg* (*Phys.*) **21,** 1–49.

WINGE, Ö. (1937) Goldschmidt's theory of sex determination in *Lymantria*, *J. Genet.* **36,** 81–89.

WINGE, Ö. and DITLEVSEN, E. (1938) A lethal gene in the Y chromosome of *Lebistes*, *C. R. Labor. Carlsberg* (*Phys.*) **22,** 203–210.

WINGE, Ö. and DITLEVSEN, E. (1947) Colour inheritance and sex determination in *Lebistes*, *Heredity* **1,** 65–83.

WITSCHI, E. (1914) Experimentelle Untersuchungen über die Entwicklungsgeschichte der Keindrüsen von *Rana temporaria*, *Arch. mikr. Anat.* **85,** 9–113; **86,** 1–50.

WITSCHI, E. (1923a) Ergebnisse der neueren Arbeiten über die Geschlechtsprobleme bei Amphibien, *Z. ind. Abst. u. Vererbungsl.* **31,** 287.

WITSCHI, E. (1923b) Über die genetische Konstitution der Froschzwitter, *Biol. Zbl.* **43,** 83–96.

WITSCHI, E. (1924) Die Entwicklung der Keinzellen der *Rana temporaria:* Urkeimzellen und Spermatogenese, *Z. Zellforsch. mikr. Anat.* **1,** 523–561.

WITSCHI, E. (1924) Die Beweise für di Umwandlung weiblicher Jungfrösche in männliche nach uteriner Überreife der Eier, *Arch. Entwicklungsmech.* **102,** 168–183.

WITSCHI, E. (1927) Sex reversal in parabiotic twins of the American wood frog, *Biol. Bull.* **52,** 137.

WITSCHI, E. (1929a) Bestimmung u. Vererbung des Geschlechts bei Tieren, *Handb. Vererbungswiss.* **2,** 115.

WITSCHI, E. (1929b) Studies on sex differentiation and on sex determination in Amphibians. II, *J. Exper. Zool.* **52,** 267–291.

WITSCHI, E. (1931) Range of the cortex-medulla antagonism in parabiotic twins of Ranidae and Hylidae, *J. Exper. Zool.* **58,** 113–145.

WITSCHI, E. (1934) Genes and inductors of sex differentiation in Amphibians, *Biol. Rev.* **9,** 460–488.

WITSCHI, E. (1935) The chromosomes of hermaphrodites. I, *Lepas anatifera*. *Biol. Bull.* **68,** 263–267.

WITSCHI, E. (1936) Studies on sex differentiation and sex determination in Amphibians. VIII, *Anat. Rec.* **66,** 483–503.

WITSCHI, E. (1956) Genic and morphologic structure of sex chromosomes. *Proceedings International Genetics Symposia*, 1956, 133–138.

WITSCHI, E. (1960) Genetic and postgenetic sex determination, *Experientia* **16,** 274–278.

WITTWER, S. H. and BUKOVAC, M. J. (1958) The effect of gibberellin on economic crops, *Econ. Bot.* **12,** 213–255.

WOLLMANN, E. L. and JACOB, F. (1959) *La Sexualité des Bactéries*. Paris, Masson.

WOLTERECK, R. (1928) Über die Populations Frederiksborger Schlosses von *Daphnia cucullata* und einige daraus neuentstandene Erbrassen, besonders diejenige des Nemi Sees, *Int. Rev. Hydrob. u. Hydrogr.* **19,** 172–203.

YAMAMOTO, TOKI-O. (1955) Progeny of artificially induced sex reversal of male genotype (XY) in the medaka (*Oryzias latipes*) with special reference to YY-male, *Genetics* **40,** 406–419.

YAMAMOTO, TOKI-O. (1958) A further study on induction of functional sex reversal in genotypic males of the medaka and progenies of sex reversal, *Genetics* **44,** 739.

YAMAMOTO, TOKI-O. (1959) The effects of estrone dosage level upon the percentage of sex reversal in genetic male (XY) of the medaka (*Oryzias latipes*) *J. Exper. Zool.* **141,** 133–153.

YAMAMOTO, TOKI-O. (1963) Induction of reversal in sex differentiation of YY zygotes in the medaka, *Oryzias latipes*, *Genetics* **48,** 293–306.

YAMAMOTO, TOKI-O. (1964) Linkage map of sex chromosomes in the medaka, *Oryzias latipes*, *Genetics* **50,** 59–64.

YANAGIMACHI, R. (1961) Studies on the sex organisation of the Rhizocephala III. The mode of sex determination in *Peltogasterella*, *Biol. Bull.* **120,** 272–283.

ZINDER, N. D. and LEDERBERG, J. (1952) Genetic exchange in *Salmonella*, *J. Bact.* **64,** 679.

ZUNARELLI, R. (1962) Il differenziamento citosessuale di tre specie di *Ophryotrocha. Boll. Zool.* **29,** 417–423.

GLOSSARY

Acyclic parthenogenesis. The capacity of reproducing exclusively by parthenogenesis in strains where amphigonic reproduction has disappeared.

Agamogony. The development of a new individual from a single asexual cell (Hartmann, 1904).

A G complex. The complex of male (A) and female (G) potencies which has been postulated for sexually reproducing organisms (Hartmann, 1930). *See* Sex realizators.

Allelogenous. Females which produce only males or only females in different batches (Vandel, 1938).

Ameiotic parthenogenesis. In which meiosis has entirely been suppressed (White, 1945). *Also* Apomictic parthenogenesis.

Amictic. Female individuals producing only male or female offspring (Storch, 1924).

Amphigony. Reproduction by fertilisation, as opposed to parthenogenesis.

Amphimixis. The mingling of paternal and maternal germplasms or genotypes by fertilisation (Weissmann, 1891) or by recombination.

Amphitoky. Production of both males and females by parthenogenesis.

Amphogenous. Producing both male and female offspring.

Anisogamy. The capacity of one population to produce small and motile (generally male) and bigger, less motile (generally female) gametes.

Apomictic parthenogenesis. *See* Ameiotic parthenogenesis.

Arrhenogenous. Producing entirely or predominantly male offspring.

Arrhenotoky. Production of males by parthenogenesis.

Automictic parthenogenesis. *See* Meiotic parthenogenesis.

Balanced hermaphrodite. A population which is composed exclusively by hermaphrodite individuals (Bacci, 1950).

Bisexual. An organism producing ripe gametes of both sexes. Also a population or generation which is composed of both male and female individuals as opposed to a unisexual parthenogenetic population or generation.

Consecutive hermaphrodite. An organism whose male and female germinal cells ripen and are shed at different times and may show distinct male and female phases.

Deuterotokous. Producing both male and female offspring.

Dioecious. Having male and female flowers on different individuals. Also having separate sexes.

Epigamic sex determination. *See* Metagamic sex determination.

Eusexual. Organisms that show a regular alternance of caryogamy and meiosis.

Genotypic sex determination. Sex is mainly determined by the genotype of the zygote or spore: a misleading notion when opposed to that of phenotypic sex determination. Also, sex is fixed as a result of syngamy or caryogamy. *See* Syngamic sex determination.

Gonochoric. Having separate sexes.

Gynandromorph or gynander. An individual of a unisexual species containing a mosaic of genetically male and genetically female cells (Goldschmidt, 1915).

Heterogonic. Cycles alternating parthenogenetic with amphigonic reproduction.

Heterothallous. Algae or fungi showing haploid incompatibility (Blakeslee, 1904) or, more loosely, separate sexes.

Hermaphrodite. An organism producing ripe gametes of both sexes.

Homogametic. Sex producing only male or female determining gametes in sex digametic organisms.

Homothallous. Algae or fungi that do not show haploid incompatibility (Blakeslee, 1904) or separate sexes.

Intersex. An individual of unisexual species whose reproductive organs and (or) secondary sex characters are partly of one sex and partly of the other (Goldschmidt, 1915) although it does not show genetically different parts.

Isogamy. The production of gametes which can not be differentiated into male and female.

Mating type. A group of ciliates or bacteria which do not conjugate among themselves but with individuals of a complementary group.

Meiotic parthenogenesis. In which meiosis takes place (White, 1945). Also Automictic parthenogenesis.

Merohermaphrodite. A population which is composed by a half hermaphrodite and a half gonochoric individuals (Bacci, this book).

Metagamic. Cycles alternating asexual with sexual reproduction.

Metagamic sex determination. The sex of the progeny is not fixed as a result of caryogamy and is therefore largely subject to environmental influences. *See also* Epigamic and Phenotypic sex determination.

Mictic. Female individuals producing male and female offspring by parthenogenesis or by amphigony (Storch, 1924).

Monocyclic parthenogenesis. A single amphigonic generation is produced in the year cycle.

Monoecious. Having male and female flowers on the same individual. Also producing gametes of both sexes.

Monofactorial sex determination. Which is apparently controlled by the back-cross mechanism as though it were based upon one gene difference. *See also* Sex digamety.

Monogeny. Production of only male or female offspring.

Parasexual. Organisms that obtain genetic recombination by means other than the regular alternance of meiosis and caryogamy (Pontecorvo, 1954). It is suggested to indicate with this name only the organisms that obtain genetic recombination through somatic crossing over (Bacci, this book). *See also* Eusexual and Protosexual.

Parthenogenesis. Reproduction from unfertilized female germ cells.

Polycyclic parthenogenesis. Two or more amphigonic generations are produced in the year cycle.

Polyfactorial sex determination. Which is controlled by multiple independent sex genes (Kosswig, 1935). *See also* Sex polygamety or Plurigamety.

Polygenic sex determination. See Polyfactorial sex determination.

Phenotypic sex determination. Sex is mainly determined by environmental conditions: a misleading motion when opposed to that of genotypic sex determination. Also, sex is supposed to be fixed independently from the result of syngamy or caryogamy. *See* Progamic and Metagamic sex determination.

Primary sex characters. Sex differences concerning gametes and gonads.

Primary sex ratio. The number of male per hundred female genotypes at zygote formation.

Progamic sex determination. The sex of the progeny is determined in the egg prior to fertilisation and even prior to meiosis.

Protandrous hermaphrodite. An organism whose male germinal cells ripen before the female germinal cells. *See* Consecutive hermaphroditism.

Protogynous hermaphrodite. An organism whose female germinal cells ripen before the male germinal cells. *See* Consecutive hermaphroditism.

Protosexaul. Organisms that obtain genetic recombination through conjugation, transduction or lysogenation (Bacci, this book). *See also* Eusexual and Parasexual.

Pseudogonochorism. Consecutive hermaphroditism where exclusively male or female gonads are normally shown, and a hermaphrodite gonad is only shown at sex reversal.

Relative sexuality. The capacity of a gamete of acting as a male or as a female when mated to different gametes (Hartmann, 1923).

Secondary sex characters. Sex characters that do not concern gametes or the gonads.

Secondary sex ratio. The number of males per hundred females at birth.

Sex. The characteristics that permit to distinguish male, female, + or –, hermaphrodite, donor and recipient individuals or clones. Also the capacity of carrying out amphimixis or genetic recombination.

Sex chromosome. A chromosome which is represented differently in the two sexes and is generally sex and sex ratio determining.

Sex cycle. The alternance of caryogamy and meiosis or any recurrent series of processes that lead to genetic recombination.

Sex determination. The mechanisms by which sex differentiation is produced.

Sex differentiation. The production of male and female, or + and − gametes, by one individual or by different individuals. Also the production of donor and recipient strains among bacteria.

Sex digamety. The capacity of one sex to produce male and female determining gametes through the sex chromosome mechanism.

Sex linked. Characters whose genes are borne by the sex chromosomes.

Sex monogamety. The supposed capacity of balanced hermaphrodite or labile gonochorists and of exclusively parthenogenetic populations of producing gametes of one kind in regard to sex determination (Bacci, 1962).

Sex plurigamety. See Sex polygamety.

Sex polygamety. The capacity of male and female or of hermaphrodite individuals to produce gametes of many different types with regard to sex determination through the mechanism of polyfactorial sex determination (Bacci, 1961).

Sex ratio. The number of males per hundred females.

Sex realizators. Sex genes or sex deciding factors which are supposed to realize the A (male) and G (female) potencies in a double system of sex determination (Wettstein, 1924).

Sex reversal. Change of sexual phase. *See also* Consecutive hermaphroditism, Pseudogonochorism.

Sexuparous. Producing male and female offspring either by parthenogenesis or by amphigony (Caullery, 1913).

Subandroecious. Prevalently male plants with a few hermaphrodite or female flowers.

Subdioecious. Plant populations where a few monoecious individuals are present among a majority of unisexual plants.

Subgynoecious. Prevalently female plants with a few hermaphrodite or male flowers.

Syngamic sex determination. The sex of the progeny is fixed as a result of caryogamy. *See also* Genotypic sex determination.

Tertiary sex ratio. The number of males per hundred females at sex maturity.

Thelygenous. Producing entirely or predominantly female offspring.

Thelytoky. Production of females by parthenogenesis.

Unbalanced hermaphrodite. A population which is composed of a majority of hermaphrodites with varying degrees of maleness or femaleness and by a minority of pure male and female individuals (Bacci, 1950).

Unisexual. Having separate sexes. *See* Dioecious and Gonochoric. Also a parthenogenetic population or generation which is composed of female individuals only.

Vegetative reproduction. The development of a new individual from a group of cells in plants and animals.

Virginiparous. Reproducing by parthenogenesis only (Caullery, 1913).

W chromosome. Some authors indicate with W the sex chromosome which, in cases of female digamety, is present in the female sex only. *See* Y chromosome.

X chromosome. The sex chromosome which is present in both sexes.

Y chromosome. The sex chromosome which is present in one sex only.

Z chromosome. Some authors indicate with Z the sex chromosome which, in cases of female digamety, is present in both sexes. *See* X chromosome.

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